HER2 Testing: Past and Present

(HER2 Testing in the Era of Changing Guidelines)

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Faculty Disclosure

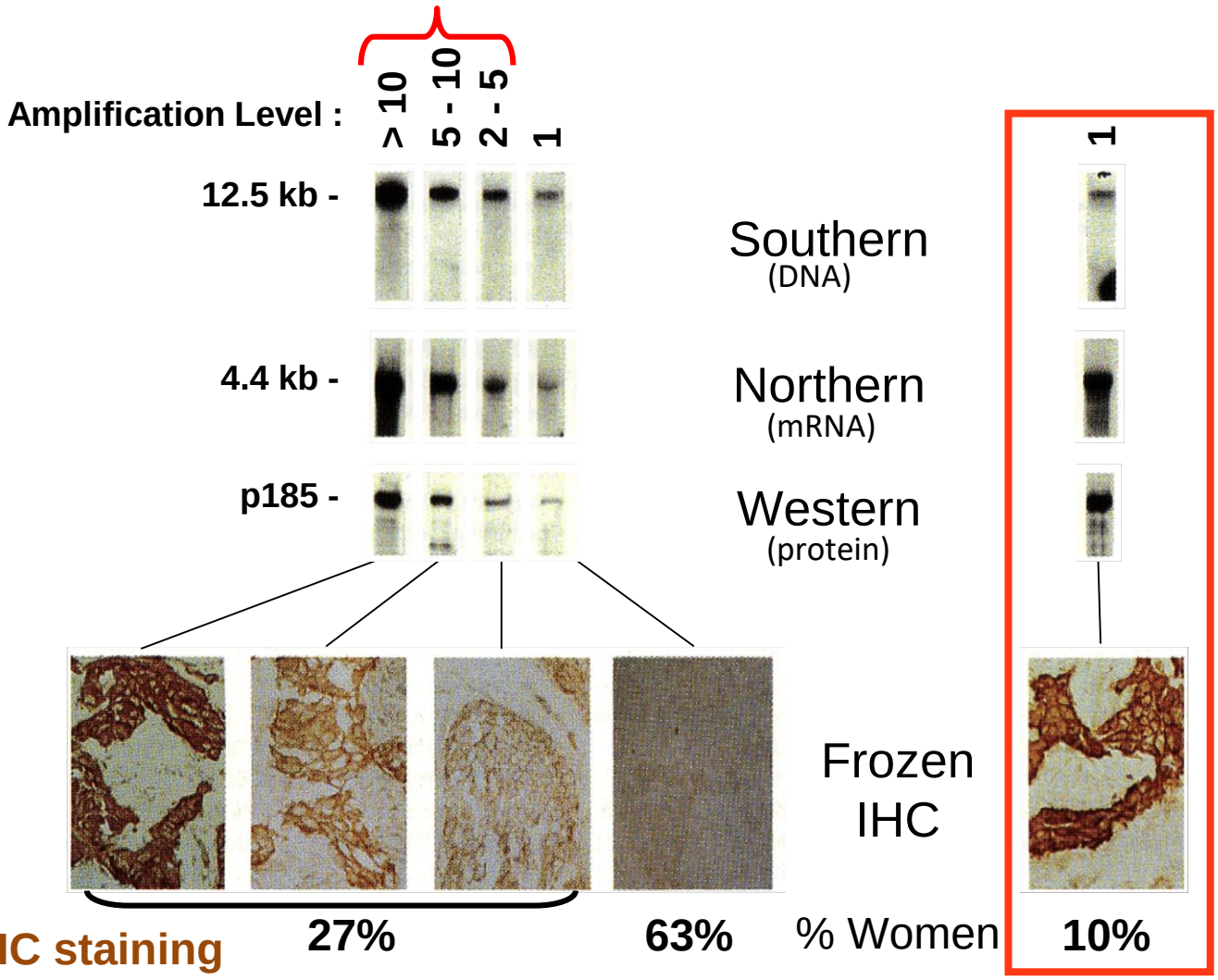
Commercial Interest	Nature of Relevant Financial Relationship	Nature of Relevant Financial Relationship	
	What was received	**For what role**	
Biocartis, SA	Honorarium	Scientific Advisory Board	
Cepheid	Research contract	Investigator	
Eli Lilly & Company	Research contract Honorarium	Investigator Scientific Advisory Board	
Zymeworks	Research contract Honorarium	Central Lab, Director Scientific Advisory Board	
Novartis Pharmaceuticals	Research contract Honorarium	Investigator Scientific Advisory Board	
Puma Biotechnology	Research contract Honorarium	Investigator Consultant	

HER2 Testing

- Background: *HER2 / ERBB2* amplification is directly correlated with HER2 overexpression in frozen tissues.
- Comparisons of ASCO-CAP Guidelines for HER2 testing (2007, 2013 / 2014 and 2018) with IHC and FISH.
- Summary of data for each ASCO-CAP FISH group according to 2013 / 2014 and 2018 guidelines.
- Assessment issues with alternative control FISH probes for HER2 “ISH-equivocal” breast cancers.
- Conclusion.

Correlation of *HER2* Gene Amplification with Overexpression

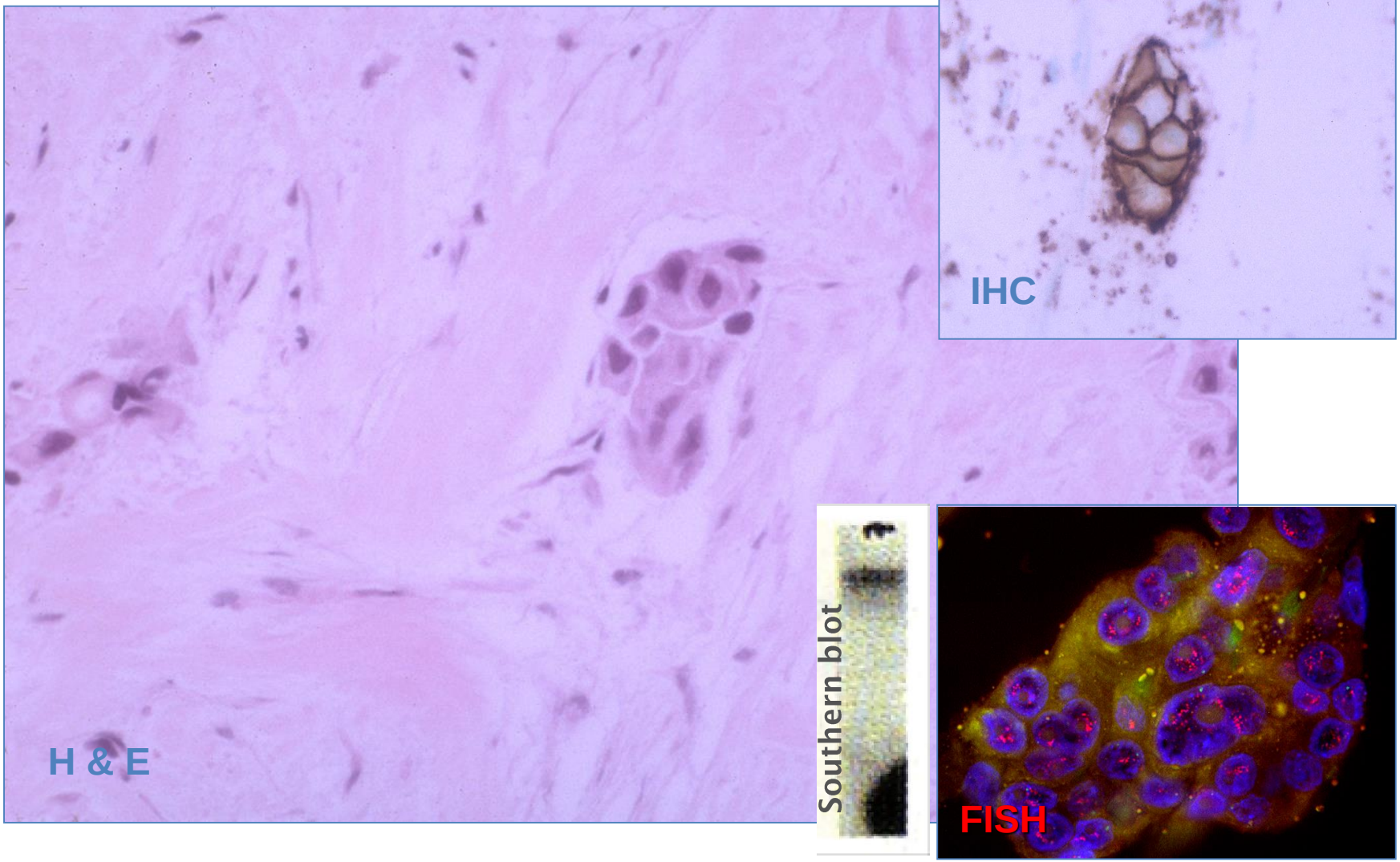
***HER2* amplification: *HER2* / *MPO* ratio ≥ 2.0**



Uniform IHC staining throughout each tumor

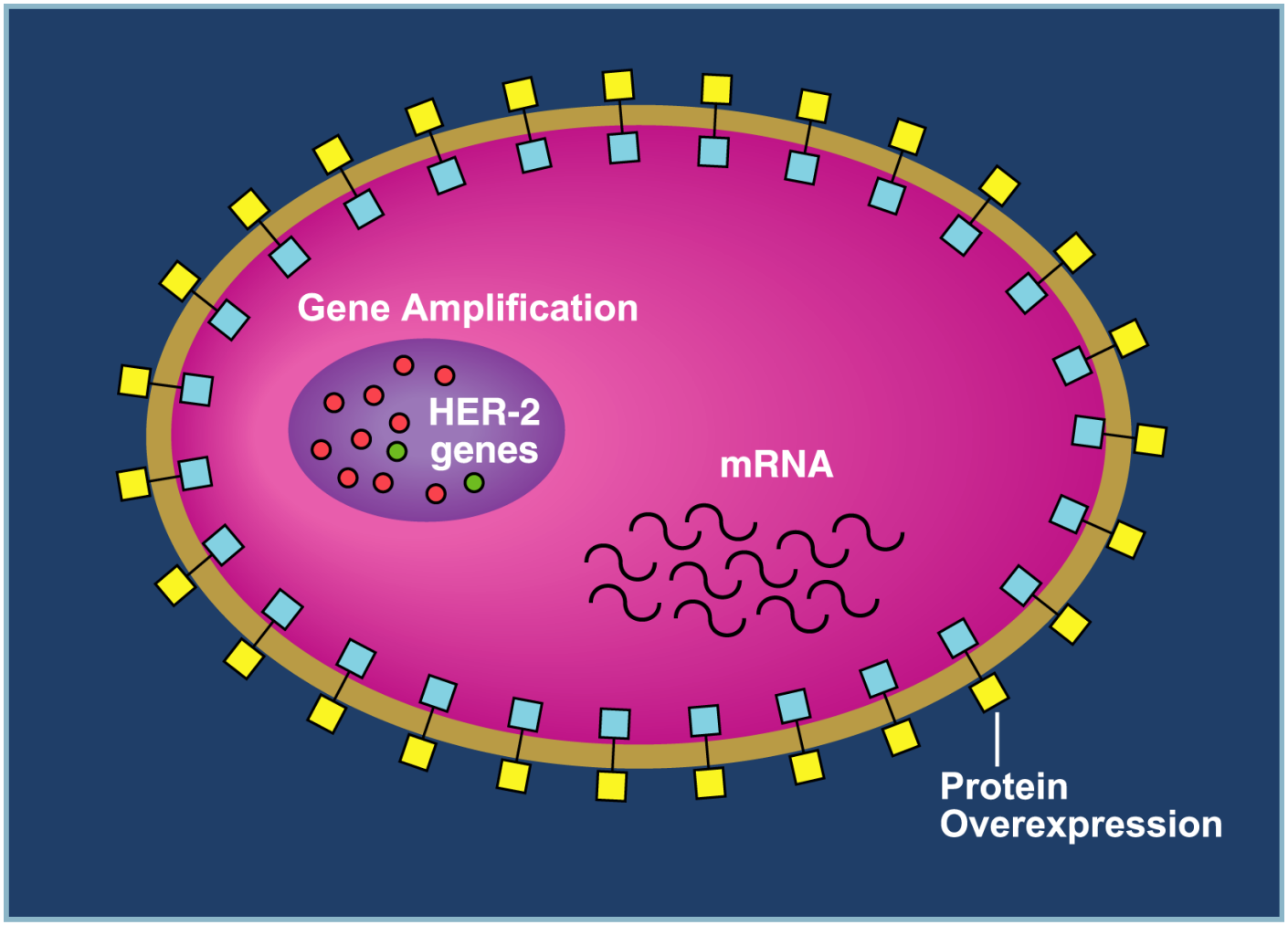
Slamon et al., *Science* 244: 707-712, 1989

Southern Blot "Single Copy or Not-amplified" Overexpression: **Actually HER2-amplified by FISH**



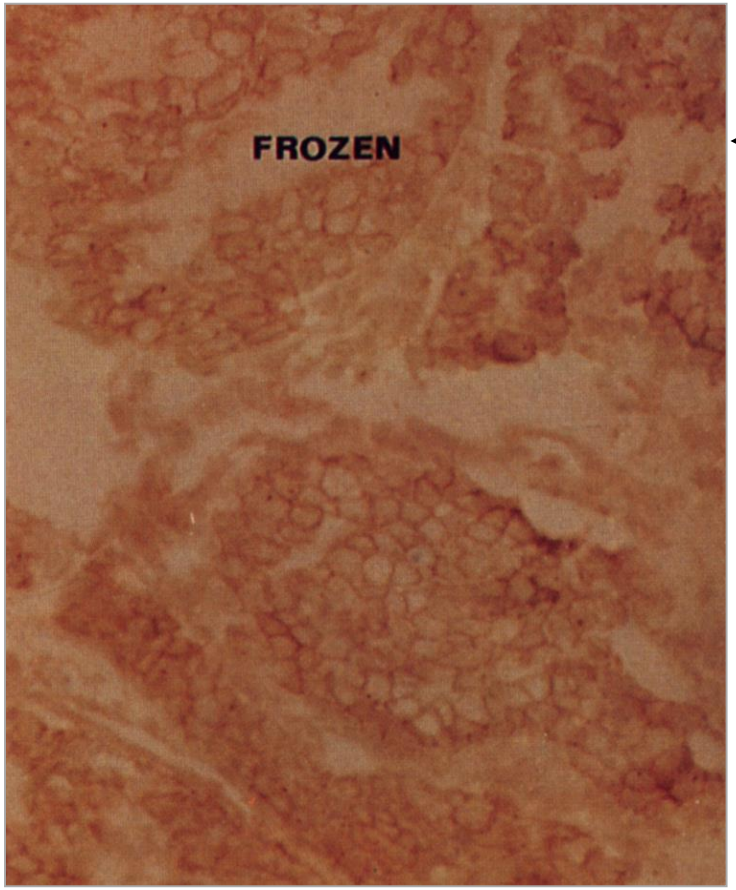
Slamon et al., Science 244:707-712, 1989; Pauletti et al., Oncogene 13:63-72, 1996

HER2 Gene Amplification is Responsible for Overexpression

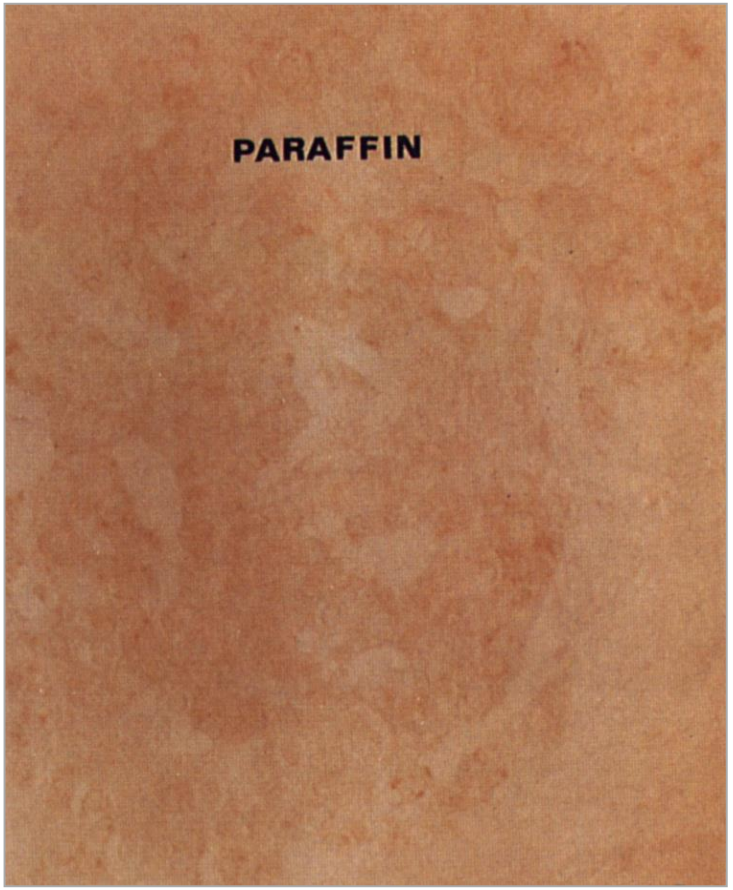


Fixation and Paraffin Embedding Result in Decreased Antigenicity (Variable False-Negatives)

2 to 5-fold *HER2* Amplified / Frozen IHC

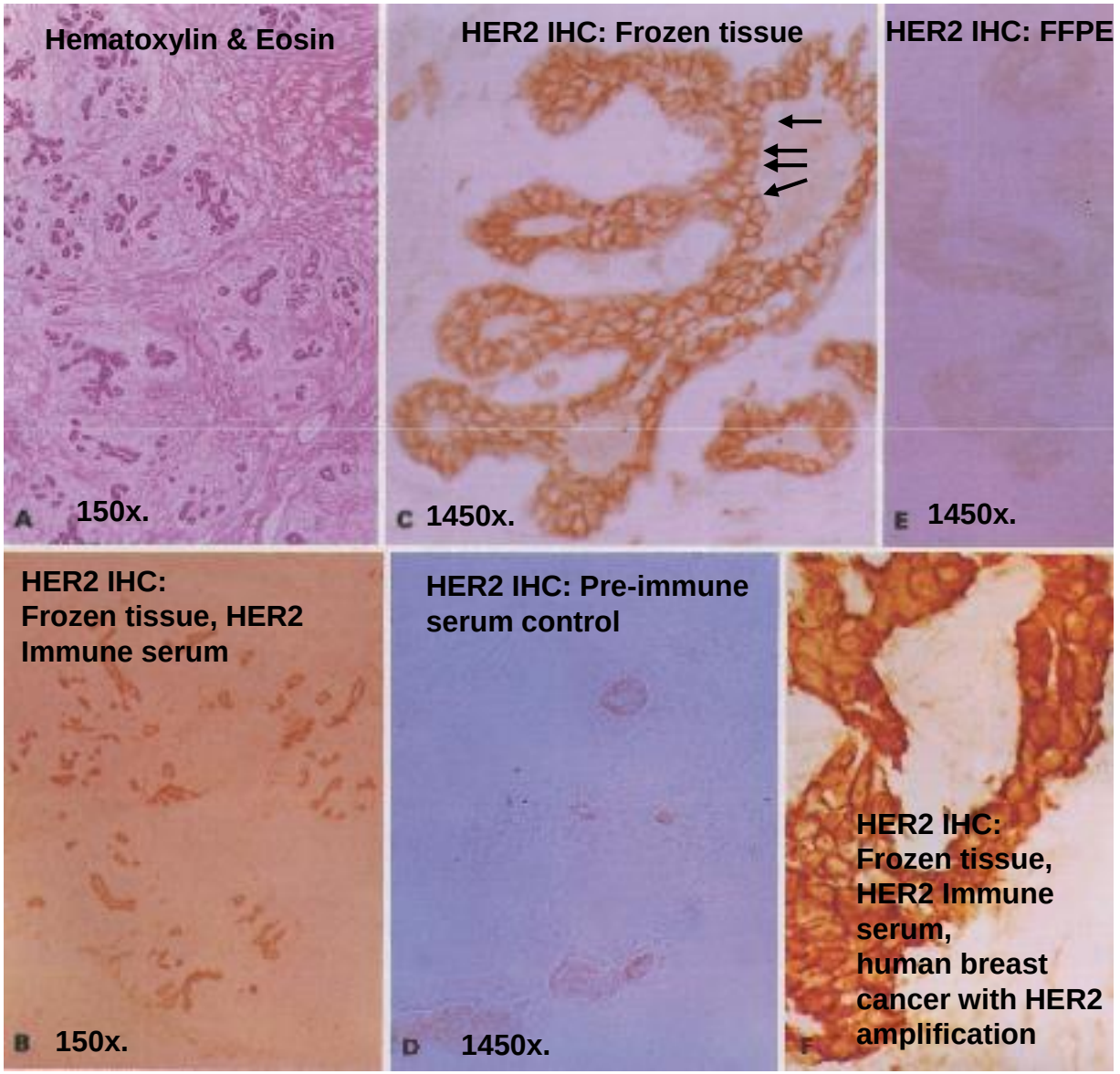


2 to 5-fold Amplified / Fixed, Paraffin IHC



Slamon et al., *Science* 244: 707-712, 1989

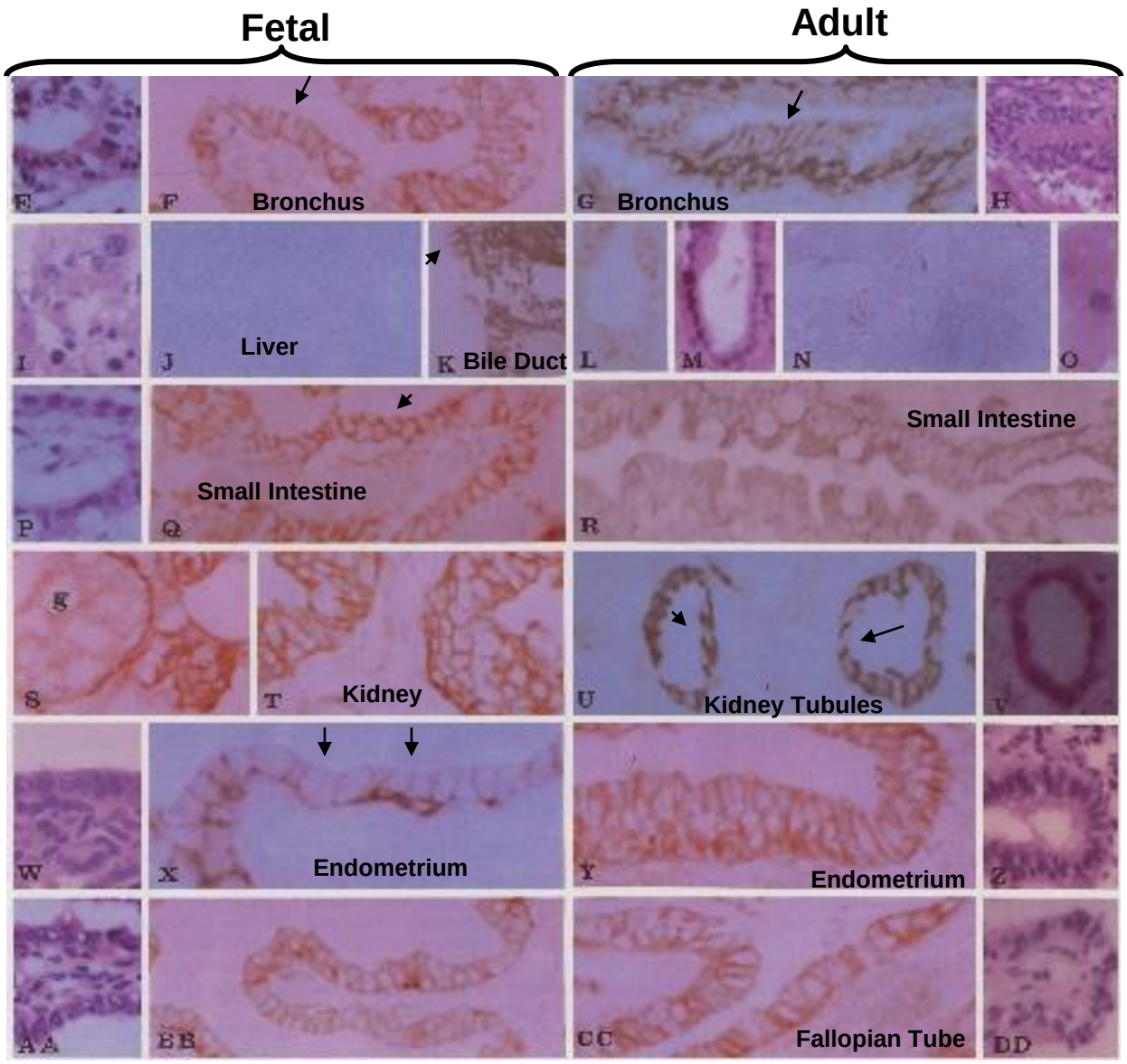
HER2 Protein Expression by IHC in Frozen Normal Breast Tissues



Press MF, Cordon-Cardo C, Slamon DJ. *Oncogene* 5: 953-962, 1990

Q 1

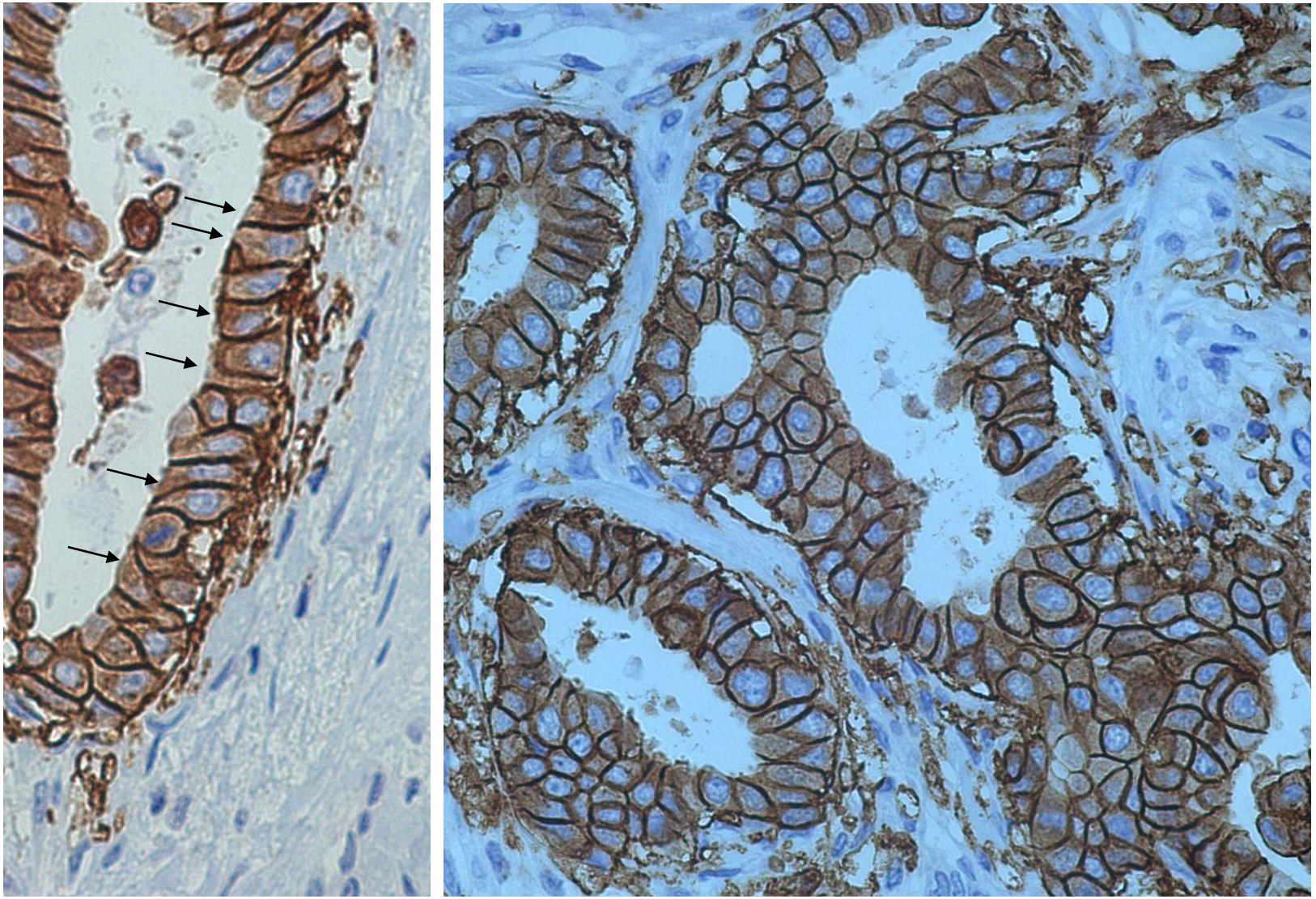
HER2 Expression in Normal Adult and Fetal Epithelium: Basal and Lateral, not Lumenal Immunostaining



Q 1

Press MF, Cordon-Cardo C, Slamon DJ. Expression of the HER-2/*neu* Proto-oncogene in Normal Adult and Fetal Tissues. *Oncogene* 5: 953-962, 1990

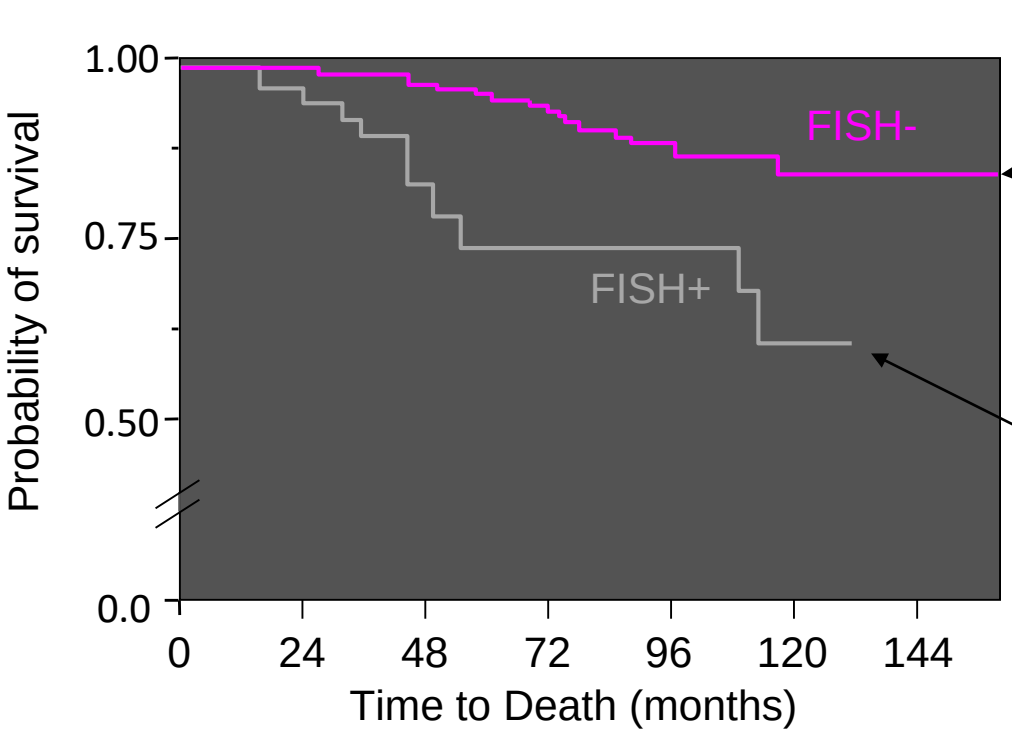
Breast Cancer: Basal and Lateral, but not Apical Membrane Staining for HER2 Protein (IHC 3+)

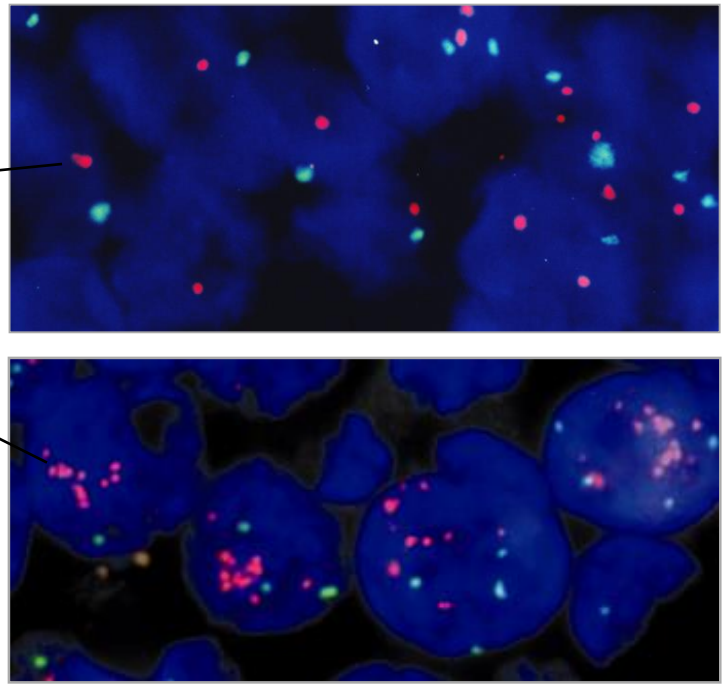


HER2 gene amplification (FISH ratio = 11.70 / 1.45 = 8.07)

Q 1

Association with Poor Outcome in Node-Negative Breast Cancer Patients





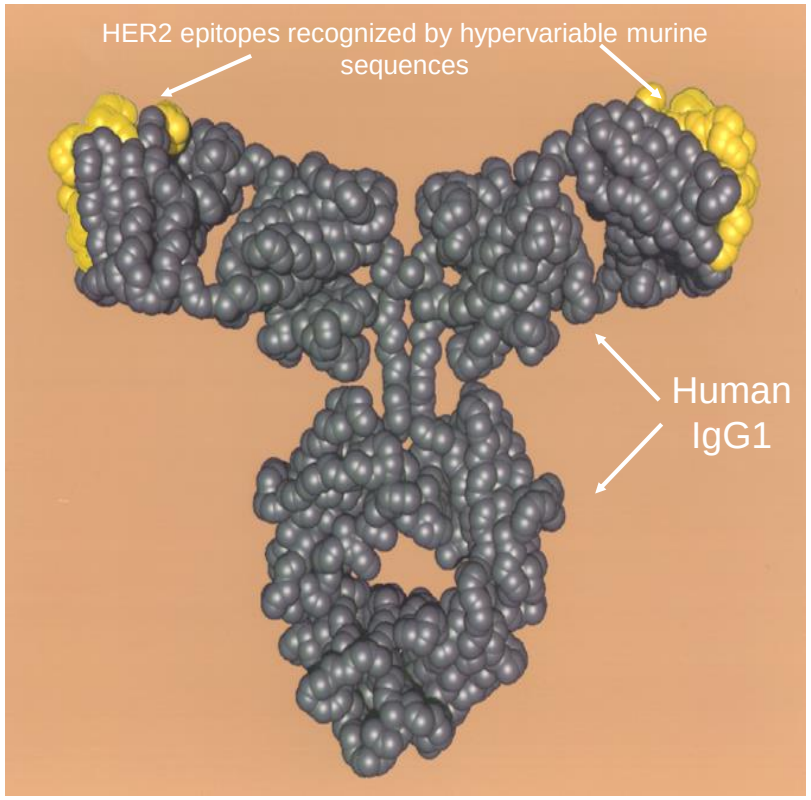
***HER2* "positive": *FISH* ratio = *HER2* / CEP17 ≥*2.0*,**

Average *HER2* gene copy number ≥4.0

Q 2

Press et al., *Journal of Clinical Oncology* 15:2894-2904, 1997

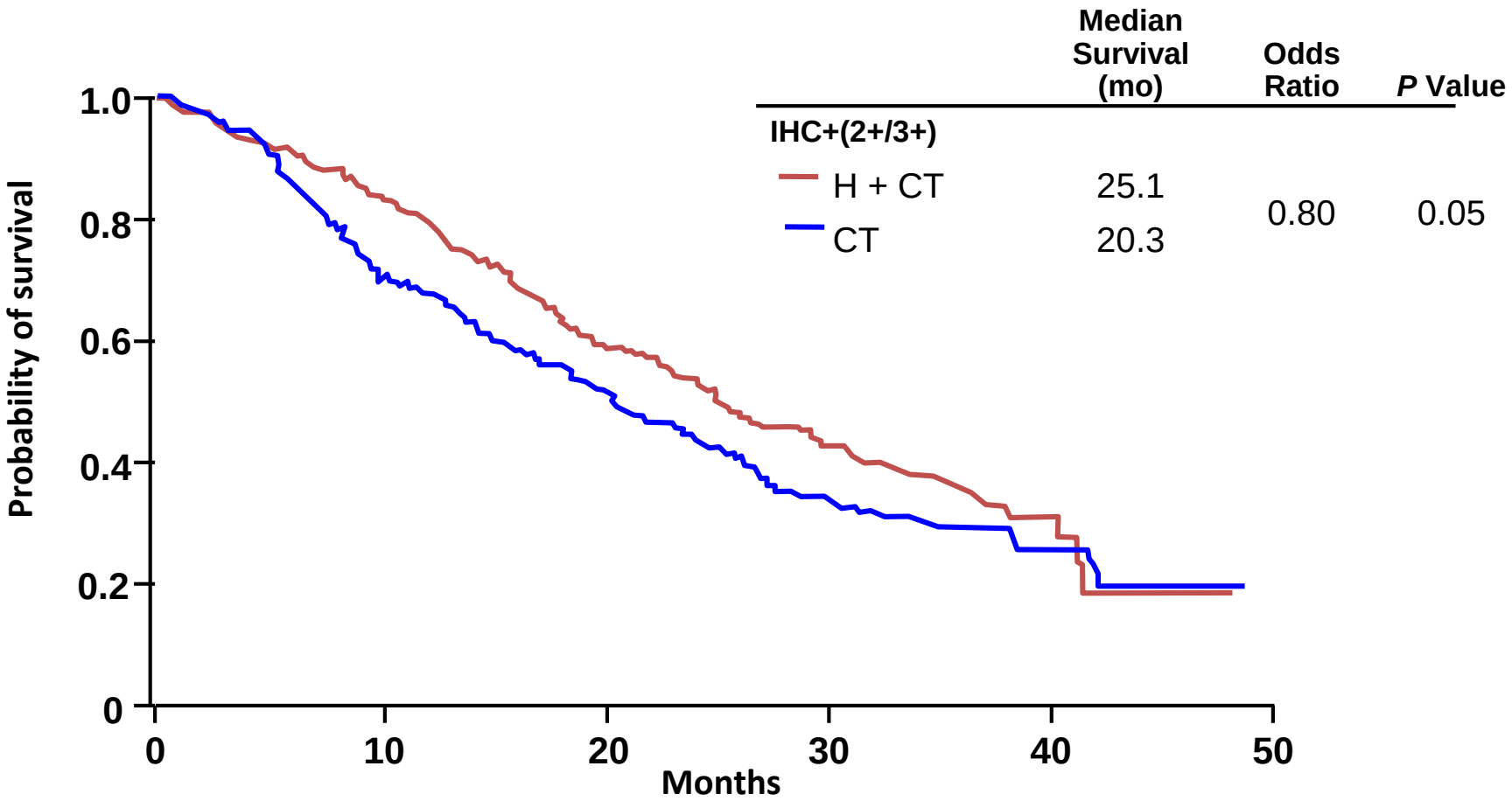
Trastuzumab (Herceptin)



Carter et al. Proc Natl Acad Sci U S A. 1992;89:4285.

- Monoclonal anti-HER2 antibody
- Humanized to:
  - Avoid immunogenicity (95% human, 5% murine)
  - Activate tumor-directed immune response
- Possible mechanisms of action:
  - Inhibition of abnormal signaling
  - Interaction (synergy) with chemotherapy
  - Enhancement of antibody-dependent cellular cytotoxicity (ADCC)

Pivotal Trial of Trastuzumab in Metastatic Breast Cancer: Association with Prolonged Overall Survival



Slamon DJ et al,
NEJM, 344:783-92, 2001.

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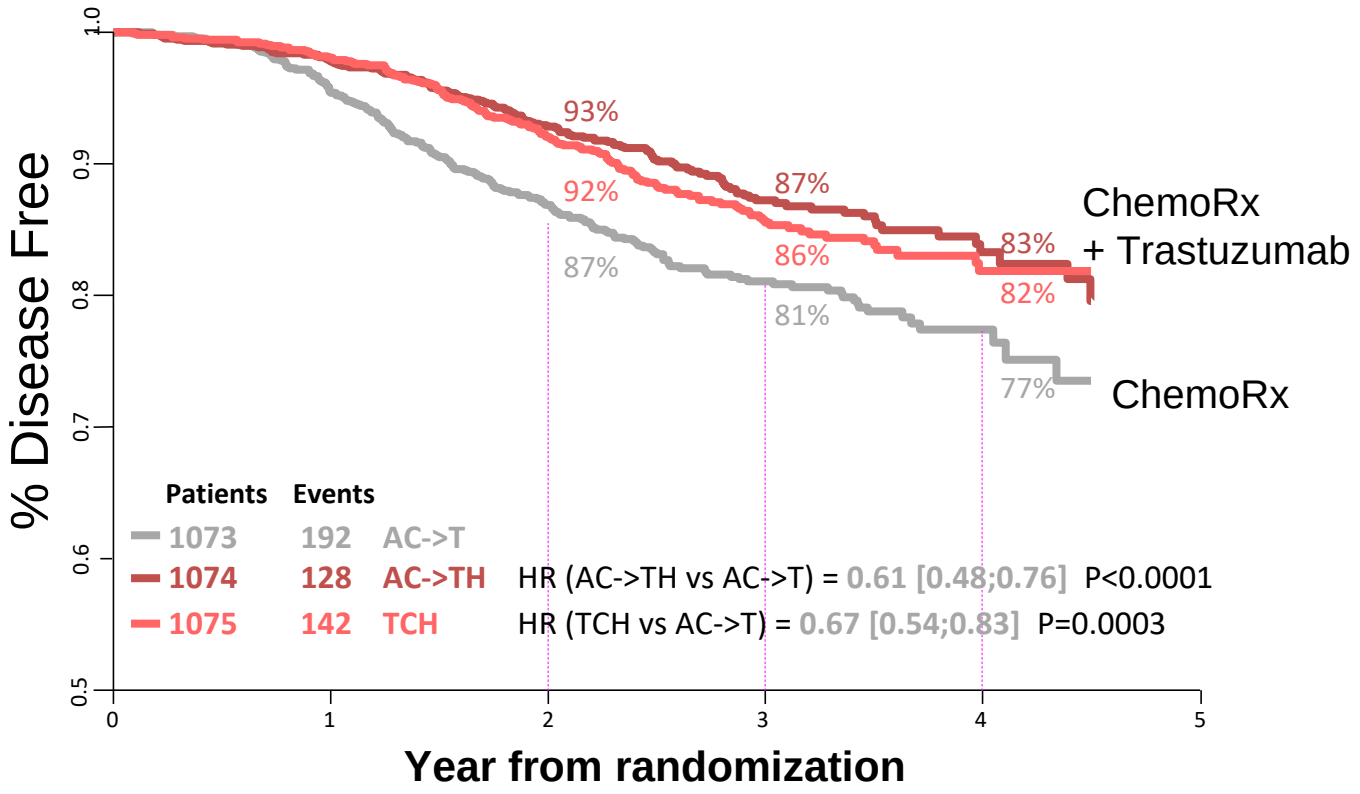
Breast Cancer International Research Group (BCIRG)-006 Trial of Adjuvant Trastuzumab in Early Breast Cancer:

Disease Free Survival



Approximately half of breast cancers were ER+ and these patients derived significant benefit from trastuzumab treatment.

Q 2

Slamon et al., NEJM, 2011

FDA-approved drugs for treatment of patients with breast cancers having *HER2* (aka *ERBB2*) amplification / overexpression

- Trastuzumab
- Pertuzumab
- TDM1 (Ado-trastuzumab emtansine)
- Lapatinib
- Neratinib

Q 3

TABLE 2.1
Companion Diagnostics Approved for HER2 Testing

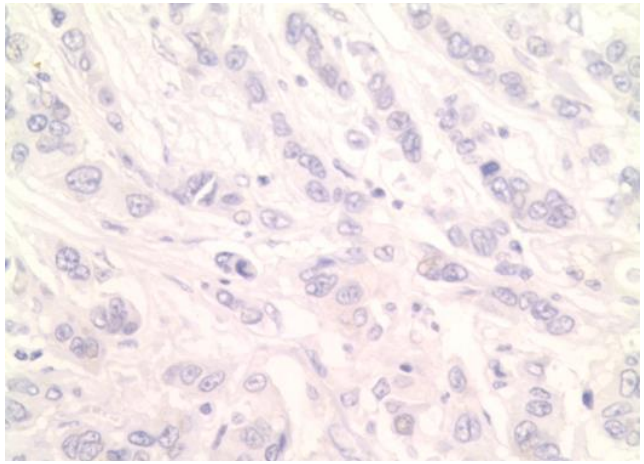
Year	Method	Assay Name	Indication	Approval Process	Company
1997	FISH	INFORM HER2[a]	Risk of early recurrence or death	Concordance Study and Cohort Study	Oncor, Inc.[a] Ventana Med Systems, Inc.
1998	IHC	HercepTest	Trastuzumab	Concordance Study with CTA	Dako, Inc.[b]
2012			Pertuzumab		
2013			Adotrastuzumab Emtansine		
2000	IHC	Pathway anti-HER2/neu (CB11)	Trastuzumab	Concordance study with HercepTest	Ventana Medical Systems[c]
2001	FISH	PathVysion	Trastuzumab	Retrospective assessment of breast cancer in trastuzumab H0648 trial compared with outcomes	Vysis, Inc.[d]
2004	IHC	InSite HER2/neu (CB11) Kit	Trastuzumab	Concordance study with HercepTest	Biogenex Laboratories, Inc.[e]
2005	FISH	Her2 FISH pharmDX Kit	Trastuzumab	Concordance Study with PathVysion and HercepTest	Dako, Inc.[b]
2012			Pertuzumab		
2013			Adotrastuzumab Emtansine		
2008	CISH	SPOT-Light HER2 CISH Kit	Trastuzumab	Concordance Study with PathVysion and HercepTest	Introgen, Inc.[f]
2011	Dual ISH	INFORM HER2	Trastuzumab	Concordance Study with PathVysion	Ventana Medical Systems
2011	Dual ISH	HER2 CISH pharmDx Kit	Trastuzumab	Concordance Study with PathVysion and PharmDx	Dako, Inc.
2012	IHC	Bond Oracle HER2 IHC	Trastuzumab	Concordance Study with PathVysion and HercepTest	Leica Biosystems
2017	NGS	FoundationOne CDx	Trastuzumab	Concordance Study with F1 LDT	Foundation Medicine, Inc.
			Pertuzumab		
			Adotrastuzumab Emtansine		

CISH, chromogenic in situ hybridization; *CTA*, clinical trials assay (4D5 and CB11); *FISH*, fluorescence in situ hybridization; *HER2*, human epidermal growth factor receptor type 2; *IHC*, immunohistochemical; *LDT*, laboratory-developed IHC test; *NGS*, next generation sequencing

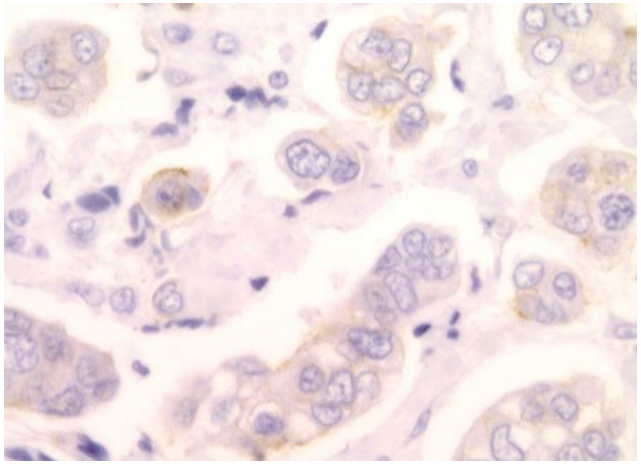
Press MF, Kim G, Khoshchehreh MMK, Ma Y, Slamon DJ. HER2 Testing in the Era of Changing Guidelines, in *HER2-Positive Breast Cancer*, Edited by Sara Hurvitz, pp 13-39, 2018

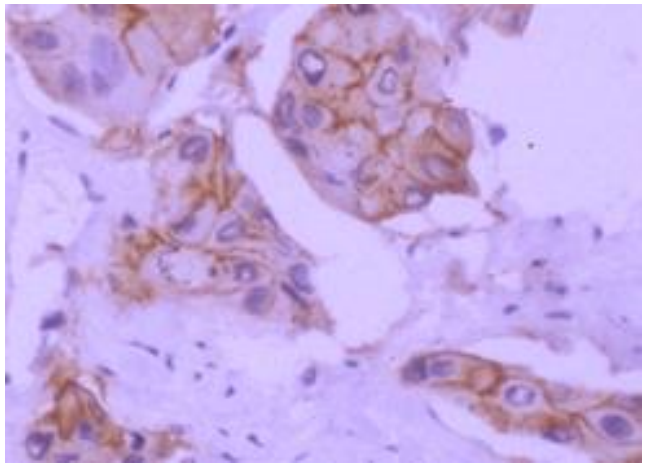
HER2 Overexpression Detection by IHC

Negative or, 0+

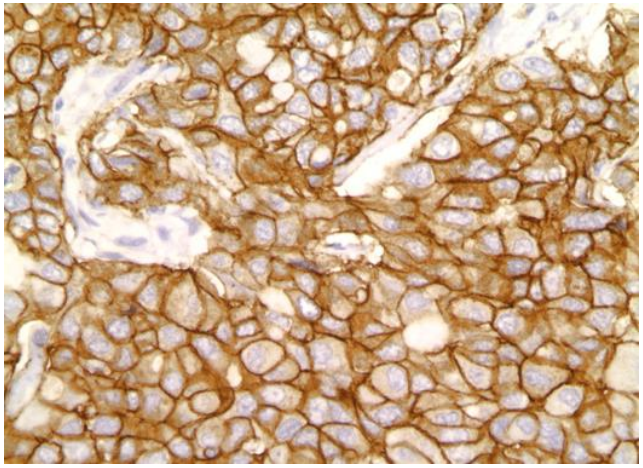


1+





2+



3+

IHC is a standard assay method in most anatomical pathology laboratories which is easily performed and easily interpreted.

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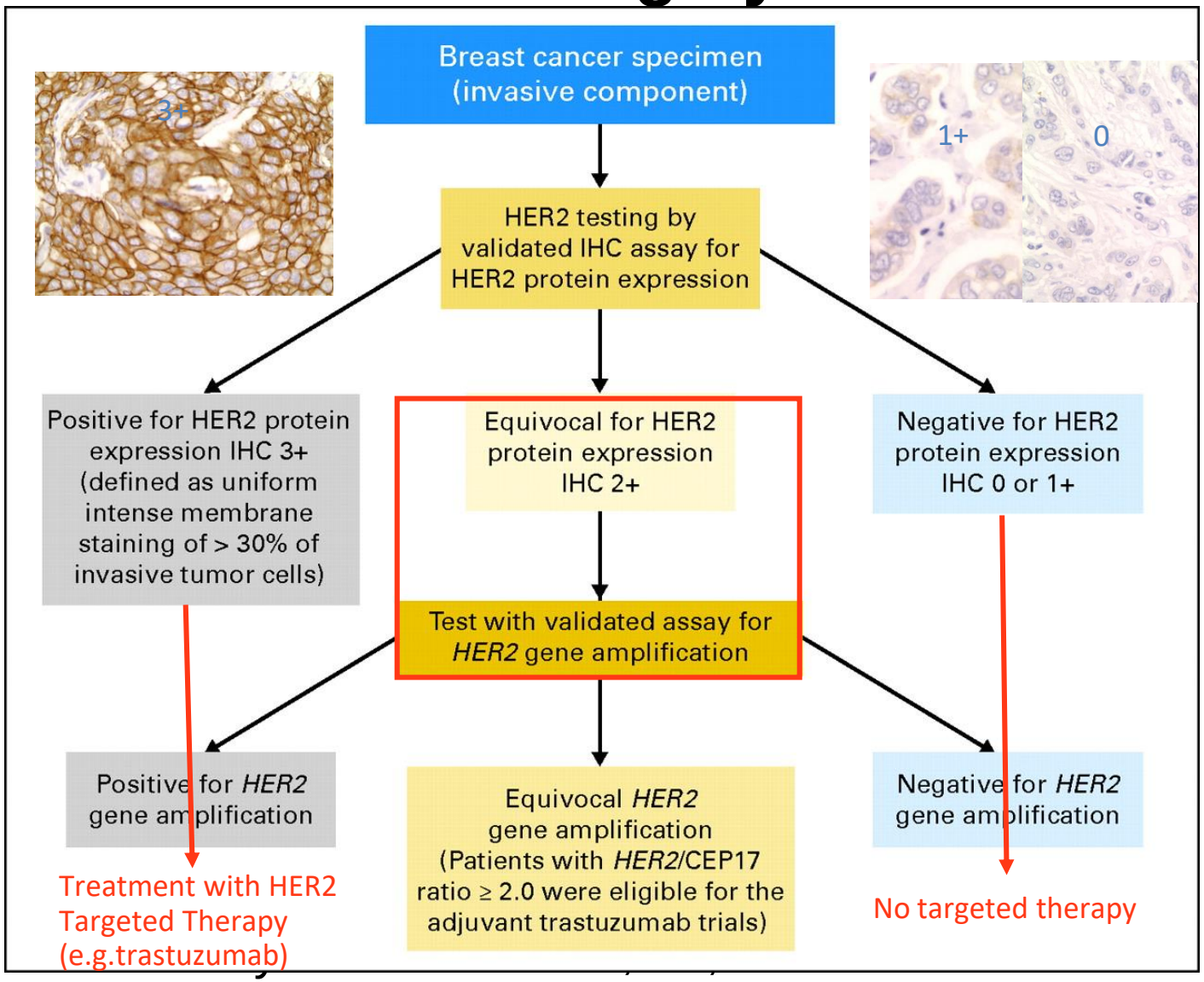
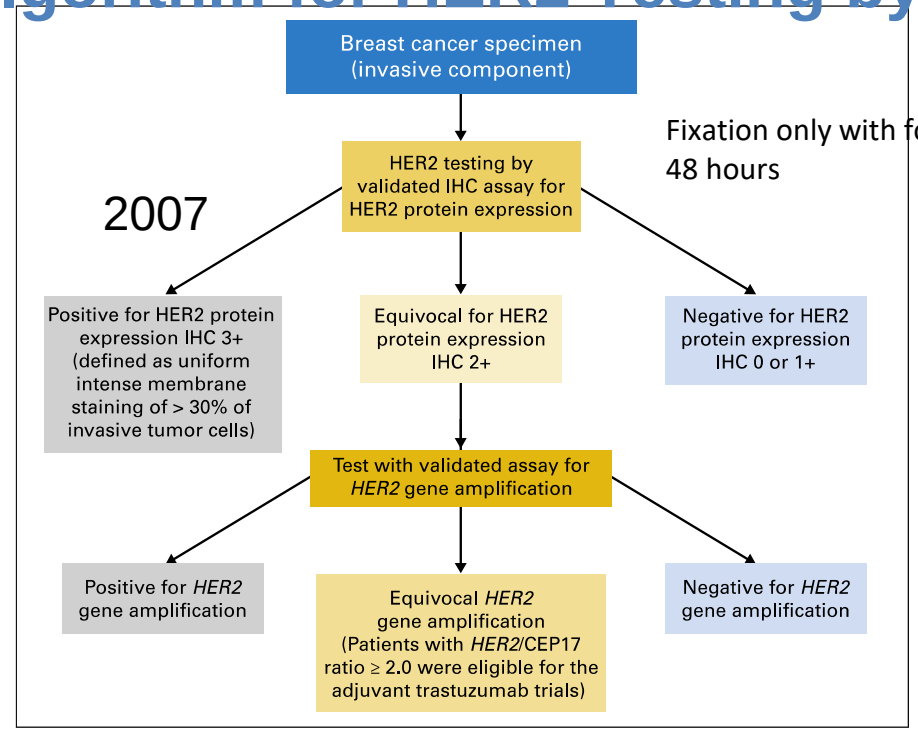
ASCO-CAP Guideline Testing Algorithm for HER2 Testing by IHC



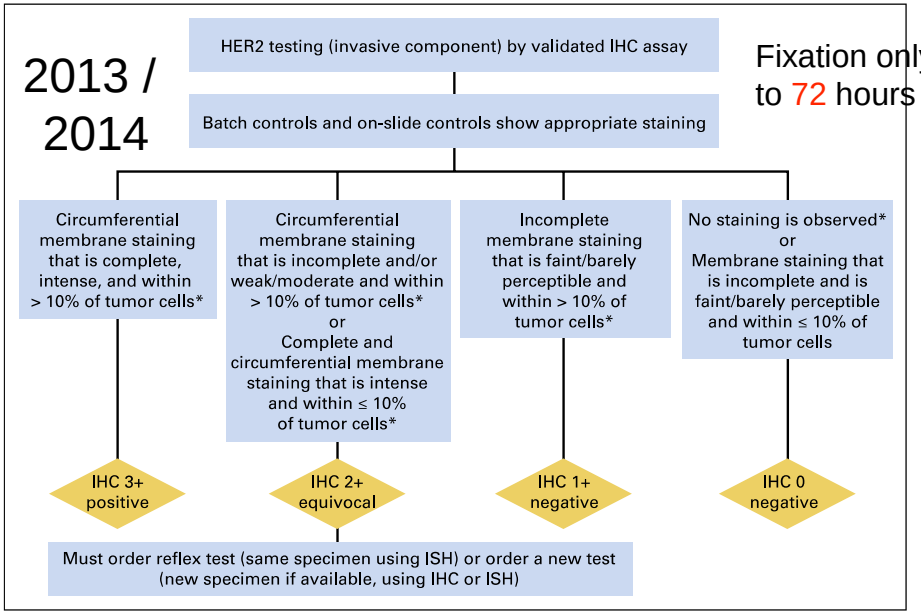
Journal of Clinical Oncology 25: 118-145, 2007

Algorithm for HER2 Testing by IHC



Changed from >10% to >30%

95% concordance required between IHC 0, 1+, 3+ and FISH to screen with IHC



Changed from >30% back to >10%

95% concordance NOT required; Lab discretion

Above: Wolff et al., *J Clin Oncol* 25: 118-145, 2007; Below: Wolff AC et al., *J Clin Oncol* 31: 3997-4013, 2013.

Q 5

Concordance between IHC and FISH: Prevalence of HER2 Gene Amplification in each IHC Category, 2008 - 2014

HER2 Gene Amplification Rate (%) in Each IHC Staining Category by Study						
IHC 0 (%)	IHC 1+ (%)	IHC 2+ (%)	IHC 3+ (%)	Number	IHC Method	Reference
0%	8.3%	22.9%	56.3%	661	**Dako HercepTest (FDA)**	Rasmussen BB et al Acta Oncol., 2008
1.6%		29.1%	86.4%	697	**A0485 (Dako)**	Grimm et al, AJCP, 2010
12.2%		66.6%	93.9%	175	**3B5 antibody (LDT)**	Panjwani et al, Indian J Med Res., 2010
3.3%		57.9%	95.2%	100	**Dako HercepTest (FDA)**	Tsuda et al, BMC Cancer, 2010*
0%	3.3%	15.2%	84.1%	200	**4B5 antibody, LDT**	Lambein et al, J Clin Pathol., 2011
0%	3.2%	21.5%	91%	681	**Dako HercepTest (FDA)**	Jorgenson JT, AJCP, 2011
12.8%		43.8%	97.8%	291	**A0485 (Dako), LDT**	Bernasconi B et al, Br Ca Res Treat., 2012
0%	23%	38.8%	100%	216	**CB11 antibody**	Martin V et al, Patholog Res Int., 2012
3.3%	7.1%	49.2%	88.4%	543	**CB11 antibody**	Lee et al, Arch Med Res., 2012
0%	12.5%	76.5%	97.3%	125	**Dako HercepTest (FDA)**	Kiyose et al, Pathol Int., 2012
2.4%		39.9%	98.1%	1437	**Dako HercepTest (FDA)**	Vergara-Lluri ME et al, Mod Pathol, 2012*
9.6%		38.9%	87.2%	396	**CB11 (Biogenix)**	Kokate P et al, Genetic Test Mol Biomark, 2012
2.6%	4.8%	28.1%	93.8%	950	**A0485 (Dako), LDT**	Park S et al, Cancer, 2012
0%	1%	19%	92%	154	**Dako HercepTest (FDA)**	Minot DM et al, AJCP, 2012
10%	5%	13%	69%	2546	**CB11 (Ventana)**	Varga Z et al, BMC Cancer, 2013
0%	2.6%	29.4%	100%	150	**4B5 (Ventana) (FDA)**	Lambein K et al., AJCP, 2013
9.4%	6.4%	13.5%	55.1%	628	**A0485 (Dako), LDT**	Fasching P et al., BCRT, 2014
1.7%	3.3%	12.4%	81.1%	2590	**Dako HercepTest (FDA)**	Schalper KA et al, Arch Pathol Lab Med, 2014

Less than 95% Concordance of IHC with FISH assay results.

Concordance between IHC and FISH: Prevalence of HER2 Gene Amplification in each IHC Category, 2014 - 2018

HER2 Gene Amplification Rate (%) in Each IHC Staining Category by Study						
IHC 0 (%)	IHC 1+ (%)	IHC 2+ (%)	IHC 3+ (%)	Number	IHC Method	Reference
0.8%	0.7%	5.8%	84.3%	1528	**Dako HercepTest (FDA)**	Varga Z et al, PLoS One, 2015
1.5%		16.4%	98.9%	811	**4B5 (Ventana)**	Green IF et al, Hum Pathol, 2015*
31.3%		50.5%	95.2%	174	**A0485 (Dako) (LDT)**	Pu X et al, Pathol Res Pract, 2015
1%	0.6%	16.8%	49.1%	3605	**Dako HercepTest (FDA)**	Morey AL, Pathology, 2016
5.6%		40.3%	100%	314	**4B5 rabbit, Ventana (FDA)**	Overcast WB et al, Virchows Arch, 2016[82]
5.8%	6.2%	36.0%	96.4%	368	**4B5 rabbit, Ventana (FDA)**	Solomon JP et al, Am J Clin Pathol, 2017
0%	3.3%	23.5%	100%	129	**4B5 rabbit, Ventana (FDA)**	Qi L, Biochem Biophys Res Commun, 2017*
4.2%		31.1%	93%	432	**Dako HercepTest (FDA)**	Eswarachary V et al, J Clin Diagn Res, 2017
3.2%		37.0%	97.8%	498	**Dako HercepTest (FDA)**	Furrer D et al, Anticancer Res, 2017*
2.5%	7.4%	31.3%	85.4%		**Averages by Studies with 4 IHC categories**	
3.9%		36.5%	91.5%		**Averages by Studies with 3 IHC categories**	

Less than 95% Concordance of IHC with FISH assay results.

Algorithm for HER2 Testing by IHC in 2018: Unchanged from 2013 / 2014

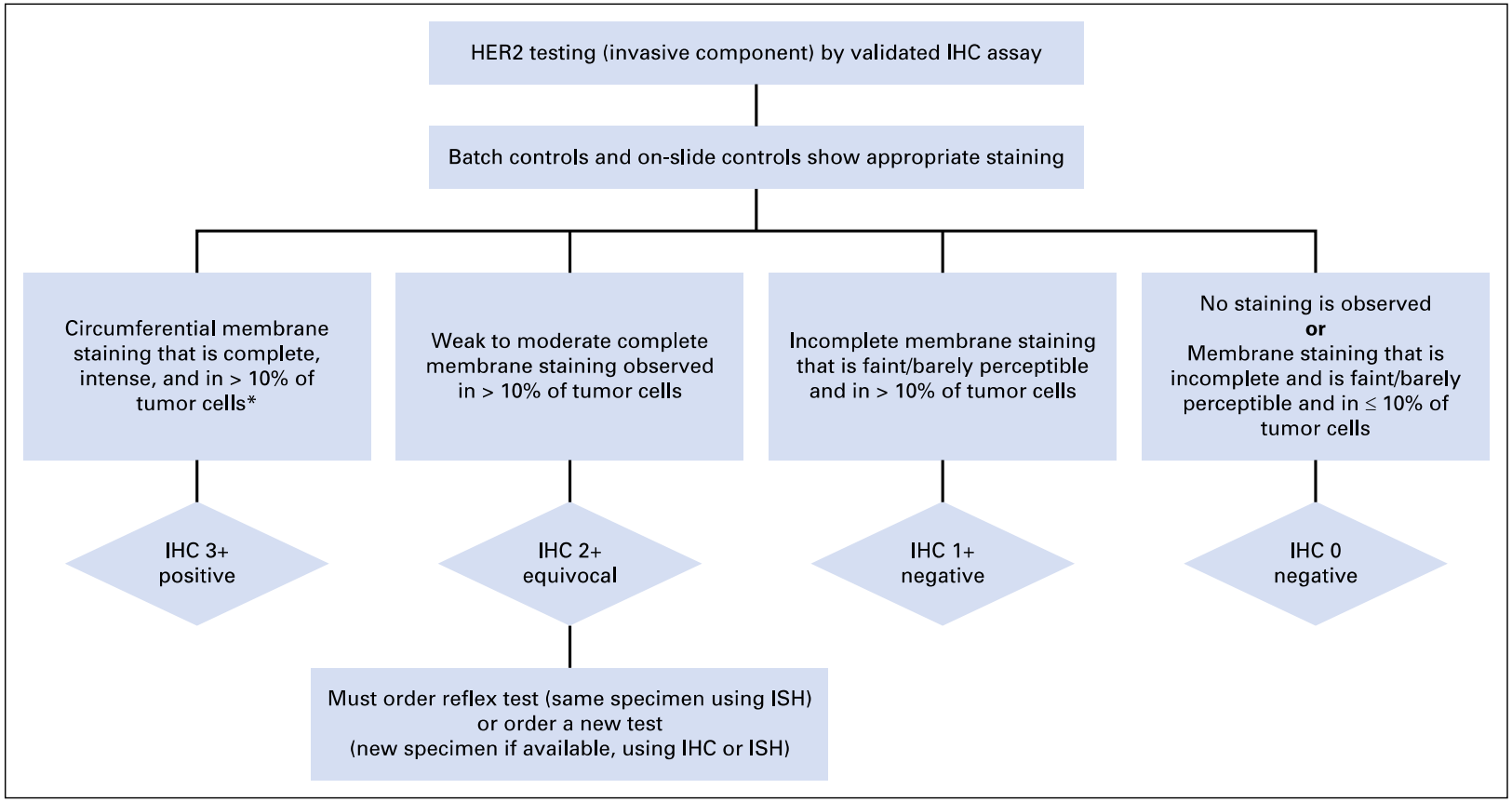


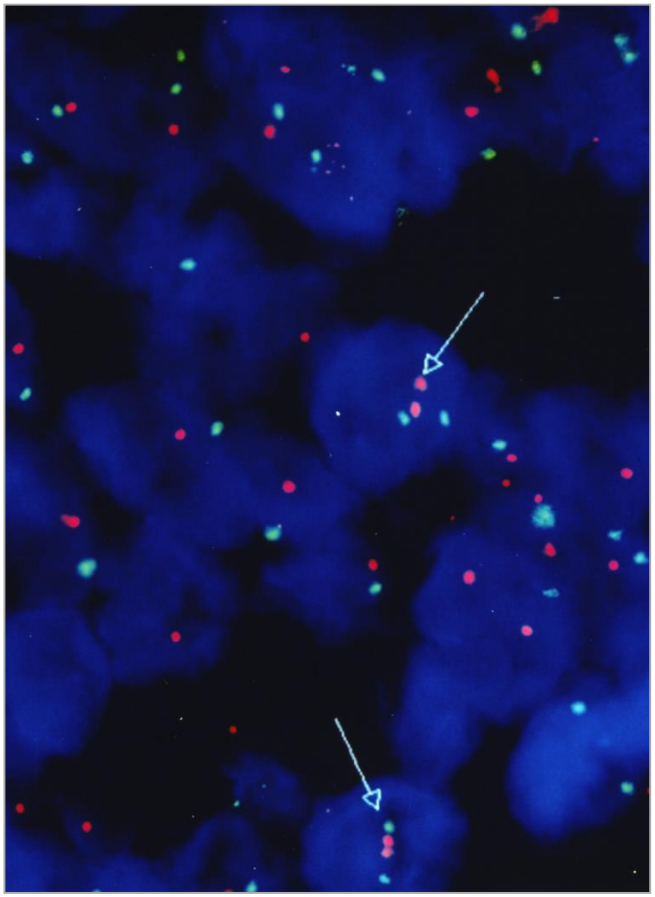
Fig 1. Algorithm for evaluation of human epidermal growth factor receptor 2 (HER2) protein expression by immunohistochemistry (IHC) assay of the invasive component of a breast cancer specimen. Note: The final reported results assume that there is no apparent histopathologic discordance observed by the 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 in ≤ 10% of tumor cells (heterogeneous, but limited in extent). Such cases can be considered 2+ equivocal, but additional samples may reveal different percentages of HER2 positive staining. ISH, in situ hybridization. (*) Readily appreciated using a low power objective and observed within a homogeneous and contiguous invasive cell population.

2007, 2013/2014 and 2018 Guidelines largely ignore both the IHC 0/1+ false-negative and the IHC3+ false-positives

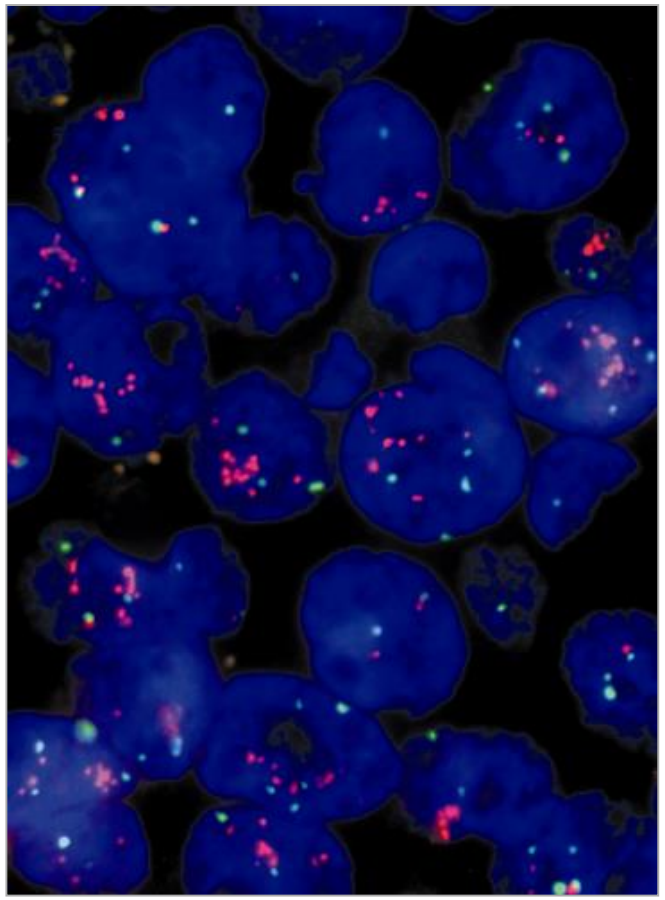
HER2 Gene Assessment by FISH

Key Features:

- Probes
  - Direct labeled
  - HER2 sequence (red)
  - Chrom 17 centromere (green)
- Interpretation
  - Signal enumeration
  - Ratio of HER2:Chr 17 signals



Ratio <2.0 Not Amplified (FISH-)

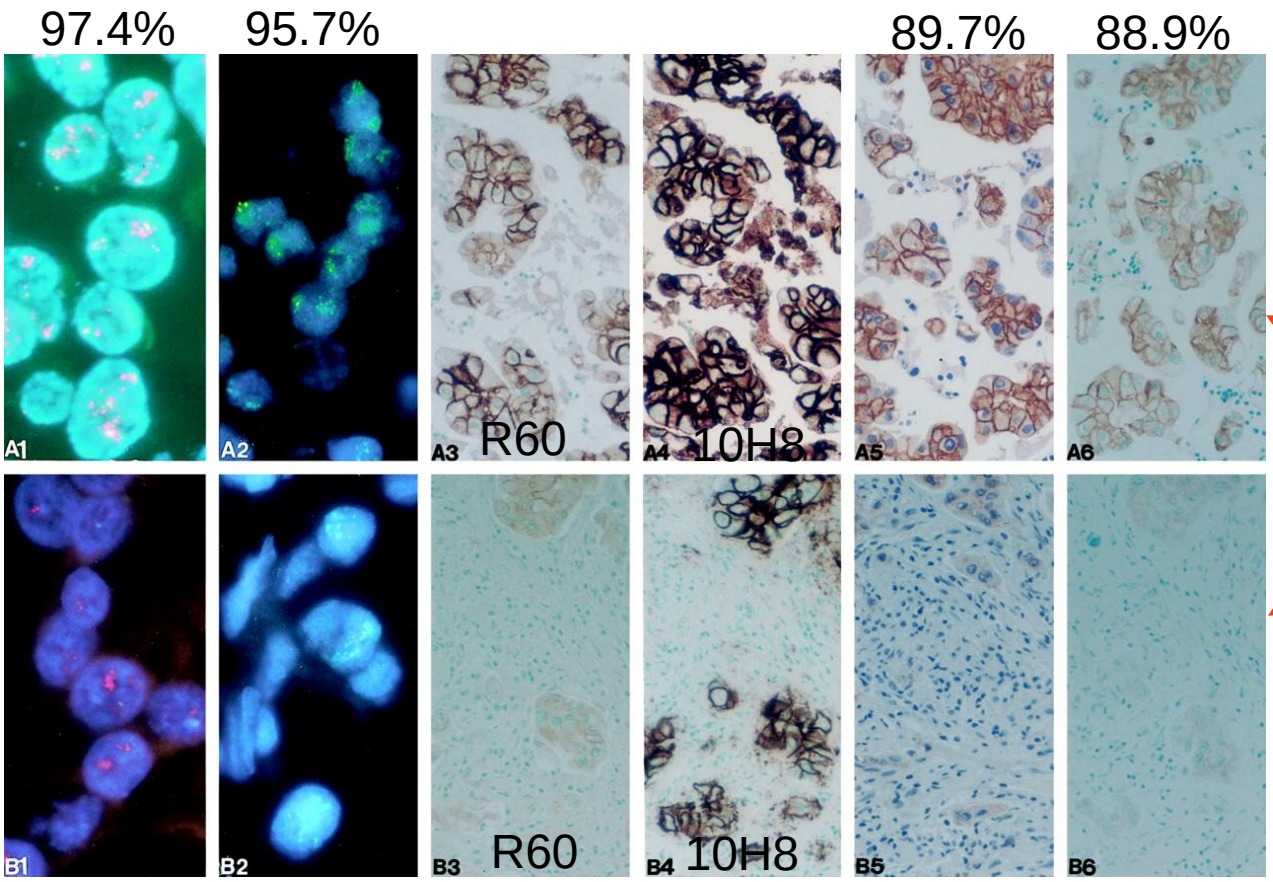


Ratio ≥2.0 Amplified (FISH+)

HER2 "positive": FISH ratio = *HER2* / CEP17 ≥2.0

Comparison of Six Different HER-2 Assays in HER2 Molecularly Characterized Breast Cancers

Concordance with Known Molecular HER2 Status



FDA-Approved FISH Assays

Lab-Dev IHC Assays

FDA-Approved IHC Assays

Molecular Status as Determined by Southern, Northern, Western blots and Frozen IHC

N = 117

Press et al., *Journal of Clinical Oncology* 20: 3095-3105, 2002

Clinical Outcome (DFS) without Trastuzumab for Patients whose Breast Cancer is HER2 IHC = 0 / 1+ and either HER2 Amplified or Not Amplified by FISH

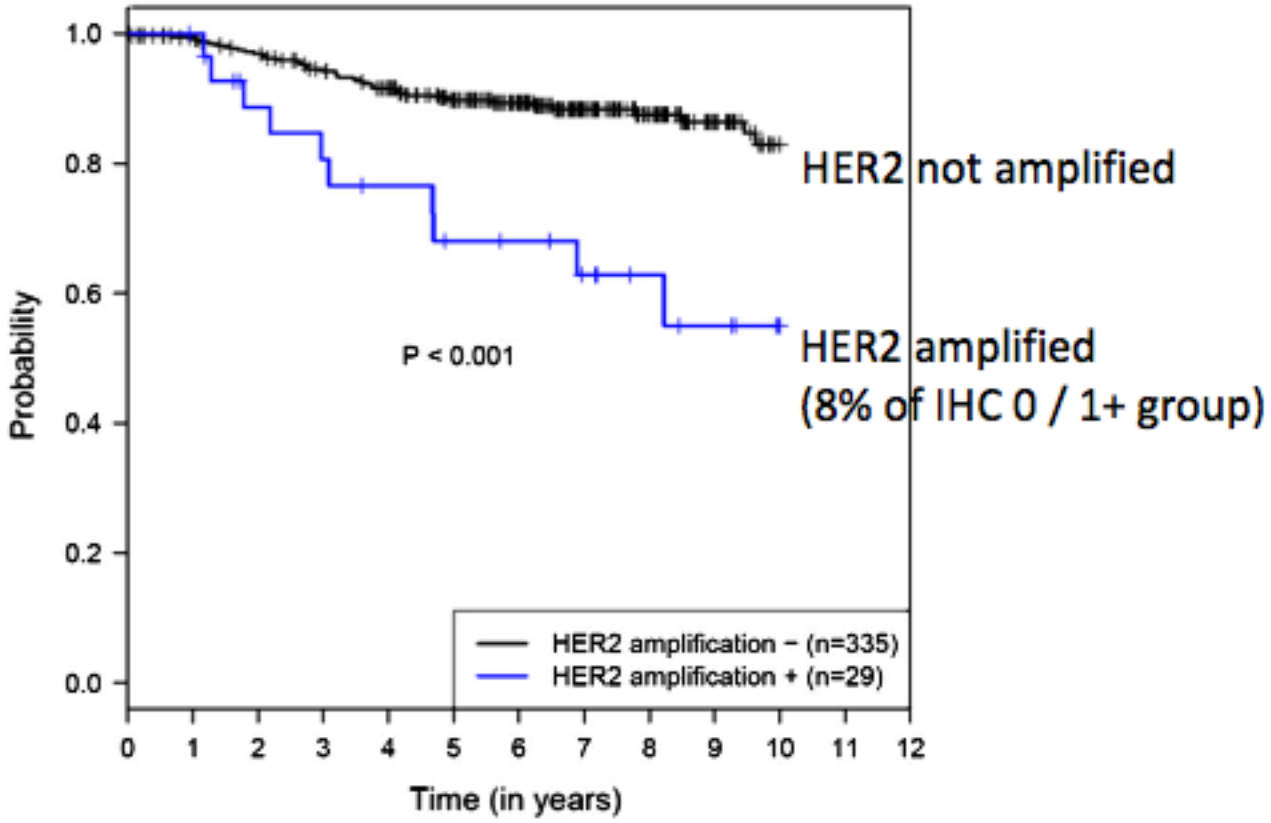


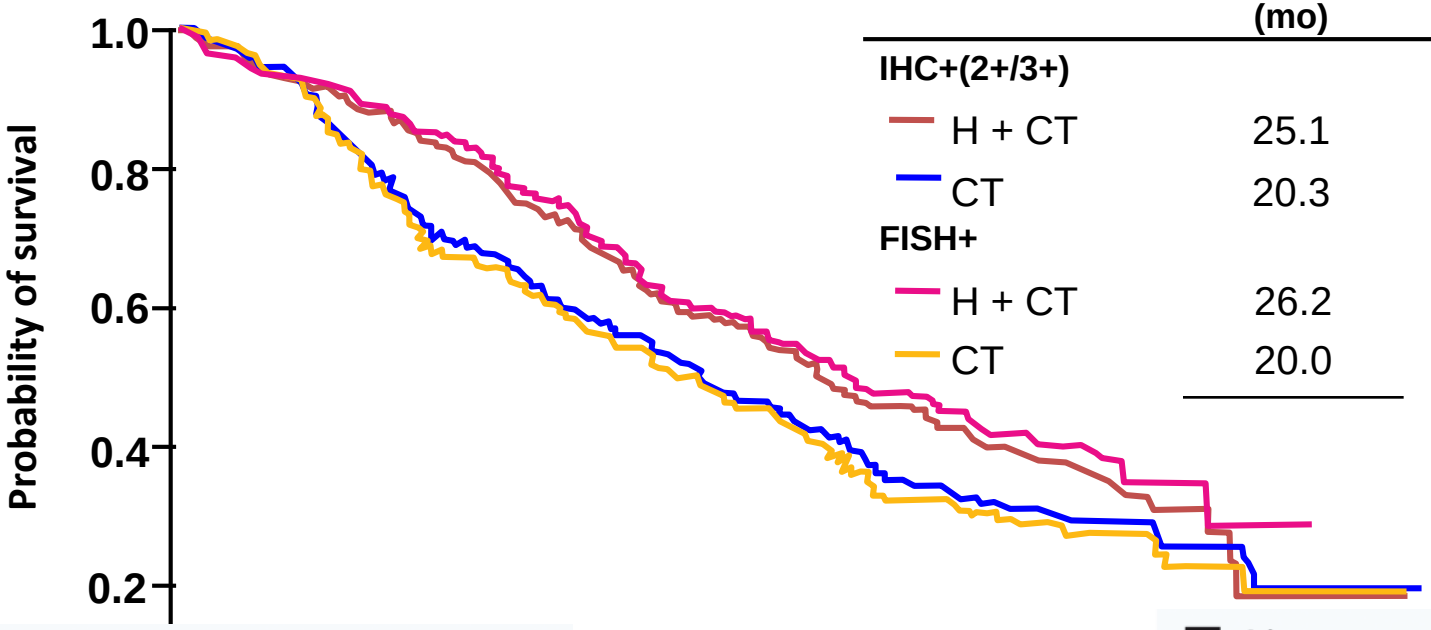
Fig. 2 Kaplan–Meier curves for distant disease-free survival relative to *HER2* assessment using fluorescence in situ hybridization in the subgroup of patients with a *HER2-I*mmunohistochemistry assessment of 0 or 1+ (restricted to patients not treated with trastuzumab)

Q 4

Fasching P, et al., *Breast Cancer Research and Treatment*, 2014

Pivotal Trial of Trastuzumab in Metastatic Breast Cancer Demonstrates the Importance of HER2 Amplification for Responsiveness

	Median Survival (mo)	Odds Ratio	*P* Value
IHC+(2+/3+)			
H + CT	25.1	0.80	0.05
CT	20.3		
FISH+			
H + CT	26.2	0.71	0.007
CT	20.0		



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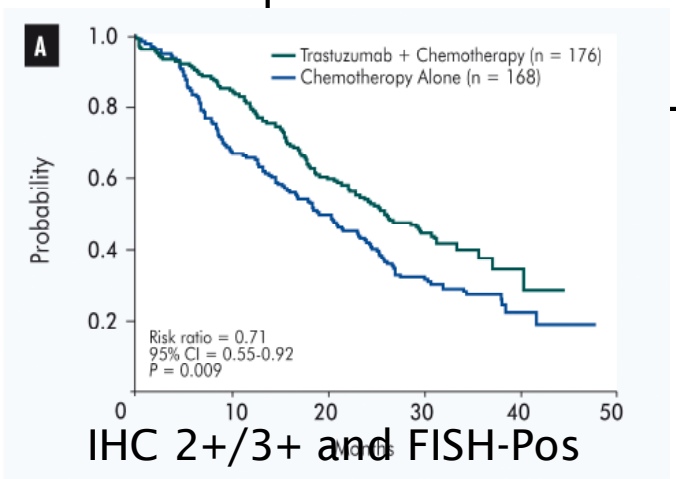
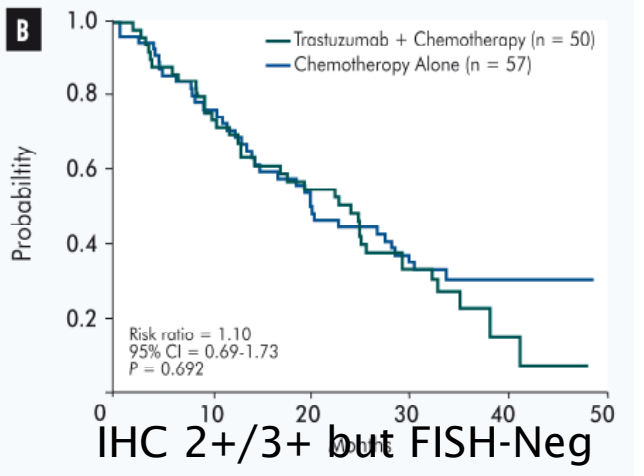


IHC 2+/3+ and FISH-Pos

Slamon DJ et al,
NEJM, 344:783-92, 2001.

Mass R, Press MF, et al.
Clinical Breast Cancer 6: 240-246, 2005



IHC 2+/3+ but FISH-Neg

ASCO-CAP Guidelines: 2013 / 2014

Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

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

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

Wolff A, et al., *Arch of Pathol Lab Invest*, 138: 241–256, 2014.

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JOURNAL OF CLINICAL ONCOLOGY

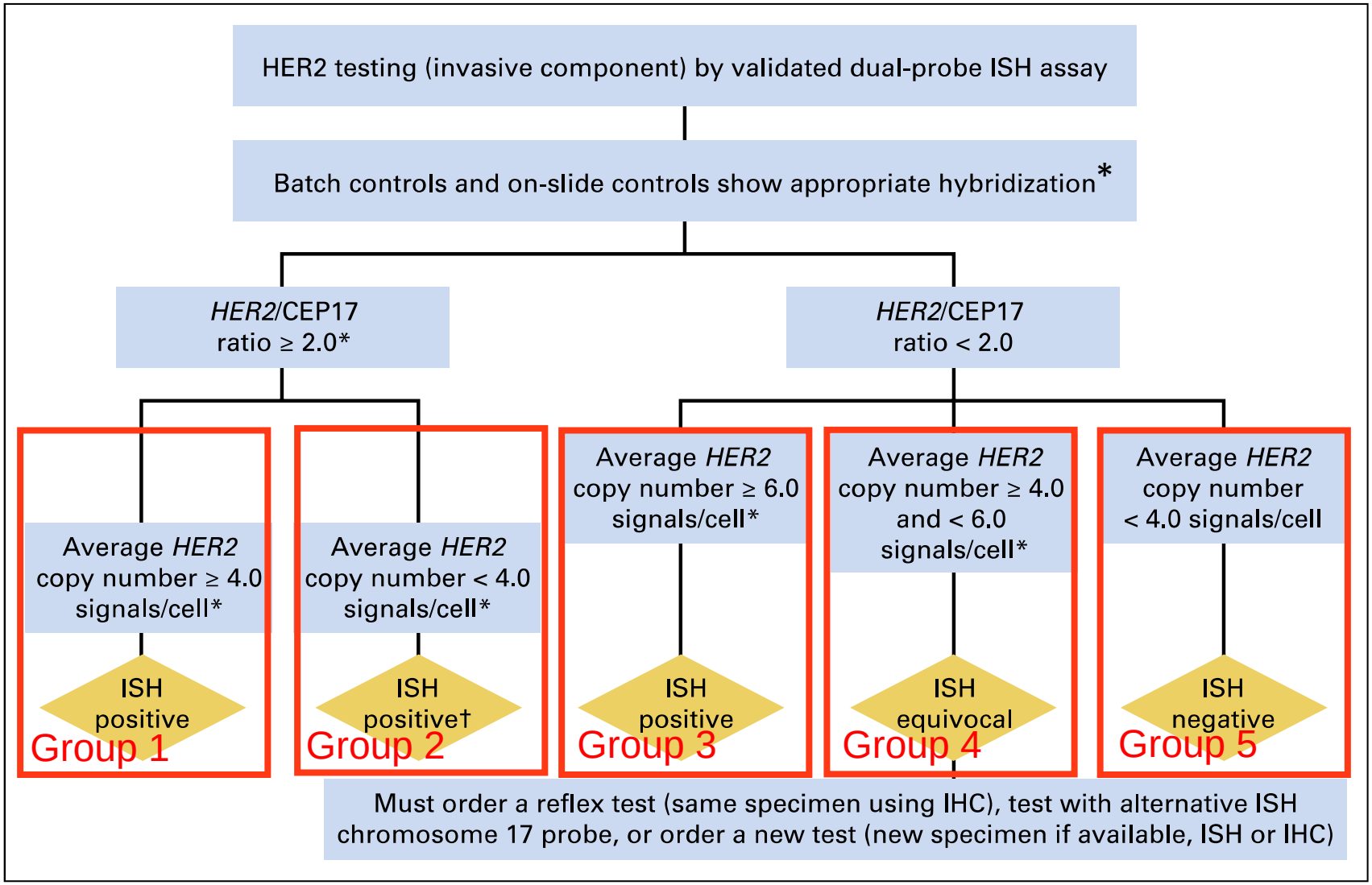
ASCO SPECIAL ARTICLE

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

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

Wolff A, et al., *Journal of Clinical Oncology*, 31: 3997-4013, 2013.

Optimal ASCO-CAP Algorithm for HER2 Testing by FISH: *HER2* probe with a control CEP17 probe



Q 5

No Published Data in 2013/ 2014.

Wolff A, et al., JCO, 2013; Arch of Pathol Lab Invest, 2014.

Study Goals

- Determine the frequency of each ASCO-CAP HER2 FISH group.
- Evaluate each ASCO-CAP FISH group for association with HER2 overexpression
- Assess ASCO-CAP groups for association with outcomes in the absence of trastuzumab and with trastuzumab treatment.

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

Experience of an Academic Consultation Practice

Michael F. Press, MD, PhD; Ivonne Villalobos, MHA; Angela Santiago, BS; Roberta Guzman; Monica Cervantes, BA; Armen Gasparyan; Anaamika Campeau, BA; Yanling Ma, MD; Denice D. Tsao-Wei, MS; Susan Groshen, PhD

N = 7,526

Archives of Pathology and Laboratory Medicine 140: 1250-1258, 2016

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

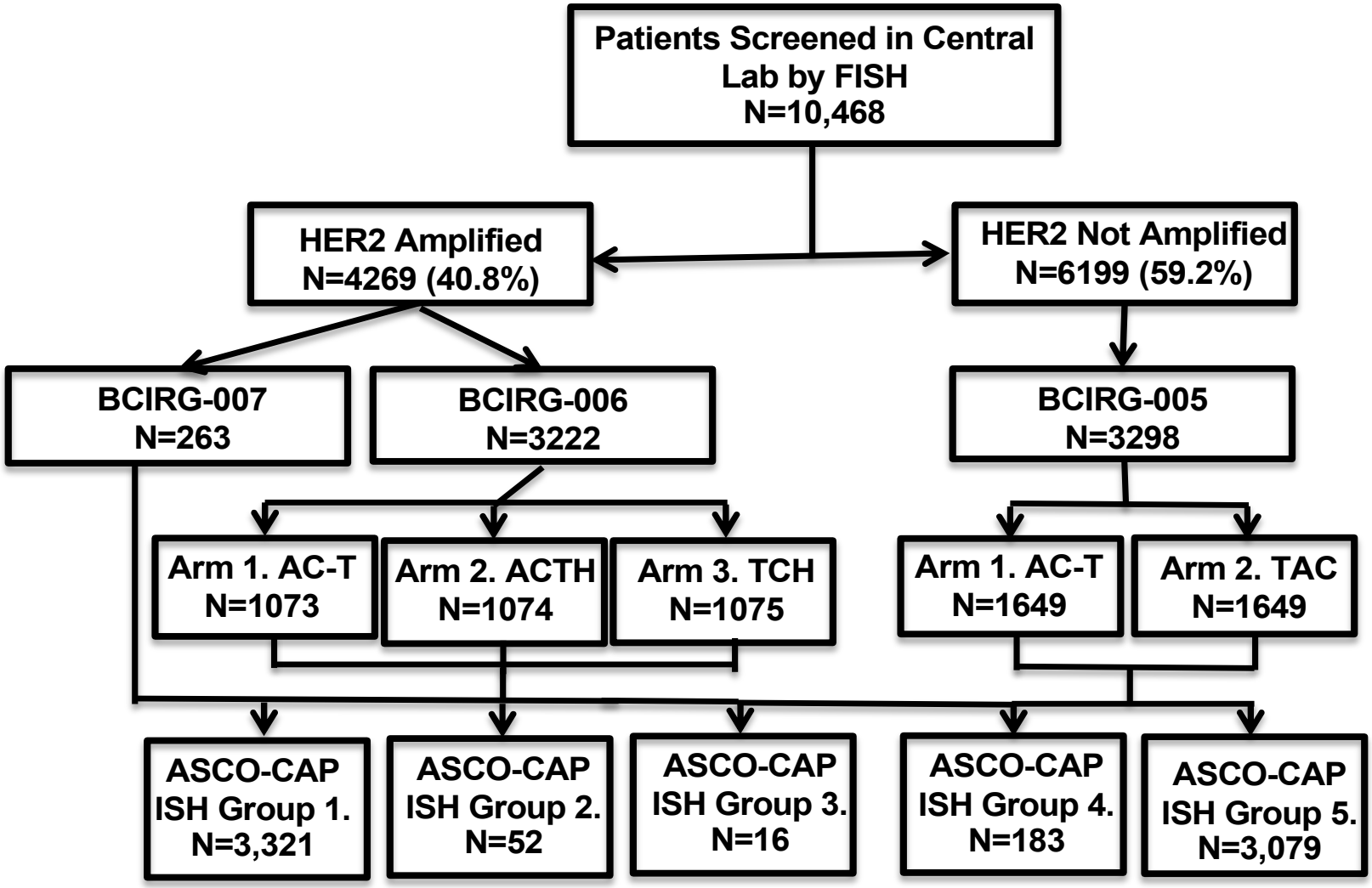
HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH): Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for Enrollment in Breast Cancer International Research Group Clinical Trials

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

N = 10,468

Journal of Clinical Oncology 34 (29): 3518-3528, 2016

Screening of Breast Cancers by BCIRG / TRIO Central Laboratory for HER2 Status: Specimen Accountability



Journal of Clinical Oncology 34 (29): 3518-3528, 2016

Assessment of HER2 by FISH According to 2014 ASCO-CAP Guidelines by Group

Group	Description of FISH category	No. of Cases	Overall %	No. of Cases	Overall %
1	Ratio ≥2.0, HER2 average ≥4.0	1328	**17.7%**	4269	**40.8%**
2	Ratio ≥2.0, HER2 average <4.0	31	**0.4%**	71	**0.7%**
3	Ratio <2.0, HER2 average ≥6.0	48	**0.6%**	55	**0.5%**
4	Ratio <2.0, HER2 average ≥4.0, <6.0	345	**4.6%**	432	**4.1%**
5	Ratio <2.0, HER2 average <4.0	5774	**76.7%**	5641	**53.9%**
Totals		7526*	**100%**	10468	**100%**
		Consultation Study		**CIRG Trials Study**	

*86 cases (1.1%) with *HER2* Genomic Heterogeneity were excluded.

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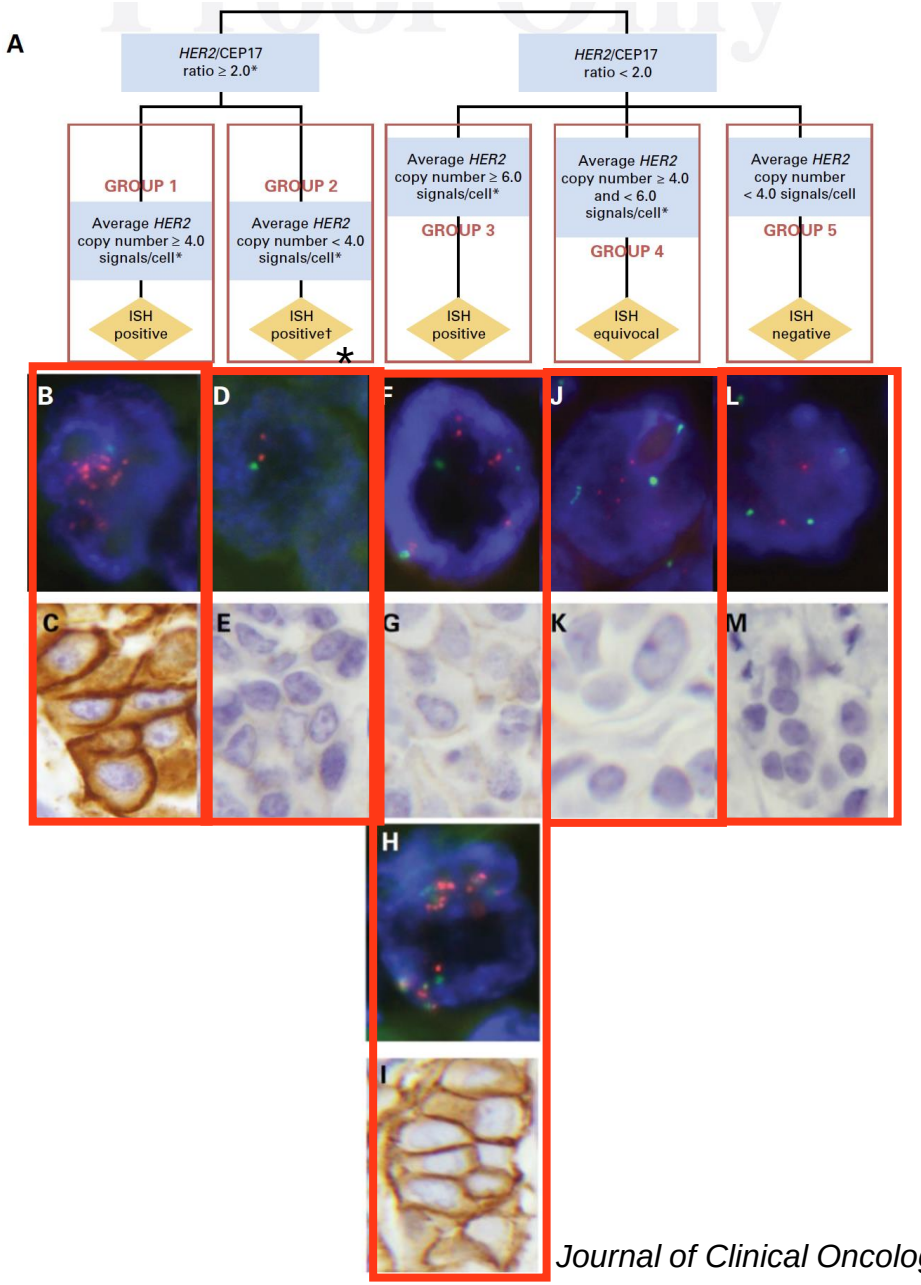
ASCO-CAP FISH Groups: Comparison of *HER2* Gene / CEP17 Status (FISH) with HER2 Protein Expression (IHC)



Journal of Clinical Oncology 34 (29): 3518-3528, 2016

ASCO-CAP FISH Groupings Compared with HER2 Protein by IHC Scores

ASCO-CAP Group	*HER2*-to-CEP17 Ratio	Average *HER2* number / cell	IHC 0 N (%)	IHC 1+ N (%)	IHC 2+ N (%)	IHC 3+ N (%)	Totals (%)	*P*-value**
Group 1	≥2.0	≥4.0	240 (11.8%)	264 (12.9%)	571 (28.0%)	965 (47.3%)	2,040	<0.0001
Group 2	≥2.0	<4.0	24 (68.6%)	8 (22.9%)	3 (8.6%)	0 (0%)	35	0.0007
Group 3	<2.0	≥6.0	5 (55.6%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	9	0.3881
Group 4	<2.0	≥4.0, <6.0	105 (78.4%)	21(15.7%)	7 (5.2%)	1 (0.7%)	134	<0.0001
Group 5	<2.0	<4.0	1,988 (94.1%)	114 (5.4%)	10 (0.5%)	1 (0.05%)	2,113	<0.0001

***IHC = immunohistochemistry; when data from both HER2 immunohistochemical assays, 10H8 and HercepTest, were available the HercepTest assay result was used.**

*******P*-value based on chi-square test for goodness of fit test of the hypothesis of equal proportions in each of the 4 IHC categories**

Journal of Clinical Oncology 34 (29): 3518-3528, 2016

ASCO-CAP Algorithm for HER2 Testing by FISH: 2018

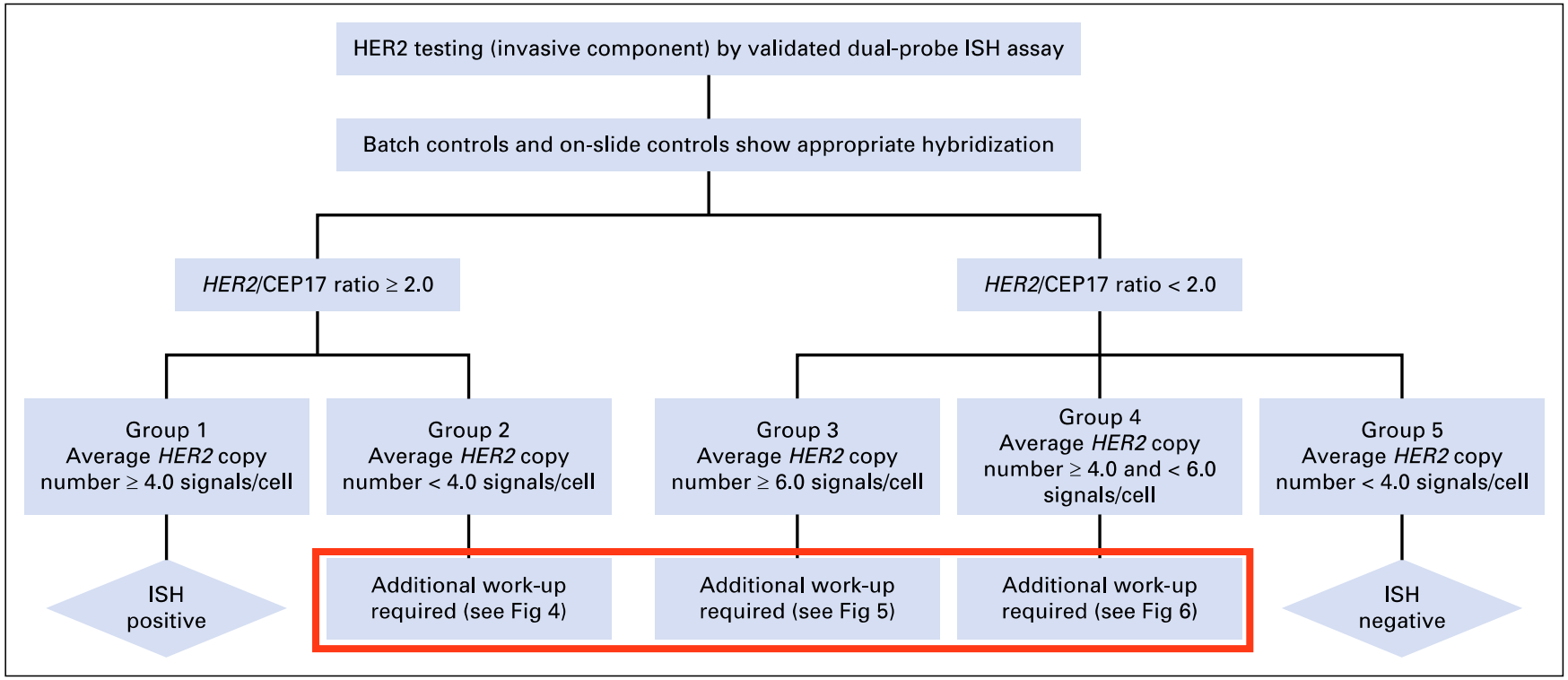


Fig 3. Algorithm for evaluation of human epidermal growth factor receptor 2 (*HER2*) gene amplification by in situ hybridization (ISH) assay of the invasive component of a breast cancer specimen using a dual-signal (*HER2* gene) assay (dual-probe ISH). Note: The final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. Regarding groups 2, 3, and 4, if not already assessed by the institution or laboratory performing the ISH test, immunohistochemistry (IHC) testing for HER2 should be performed using sections from the same tissue sample used for ISH, and the slides from both ISH and IHC should be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant assessment). CEP17, chromosome enumeration probe 17.

Wolff A, et al., *JCO*, 2018

No published data related to "problematic issues" with chromosome 17 "alternative control probes" (e.g. *TP53*, D17S122, SMS, *RARA*, *TOP2A*) **for ISH**

Q 7

Evaluation of FISH Group 2

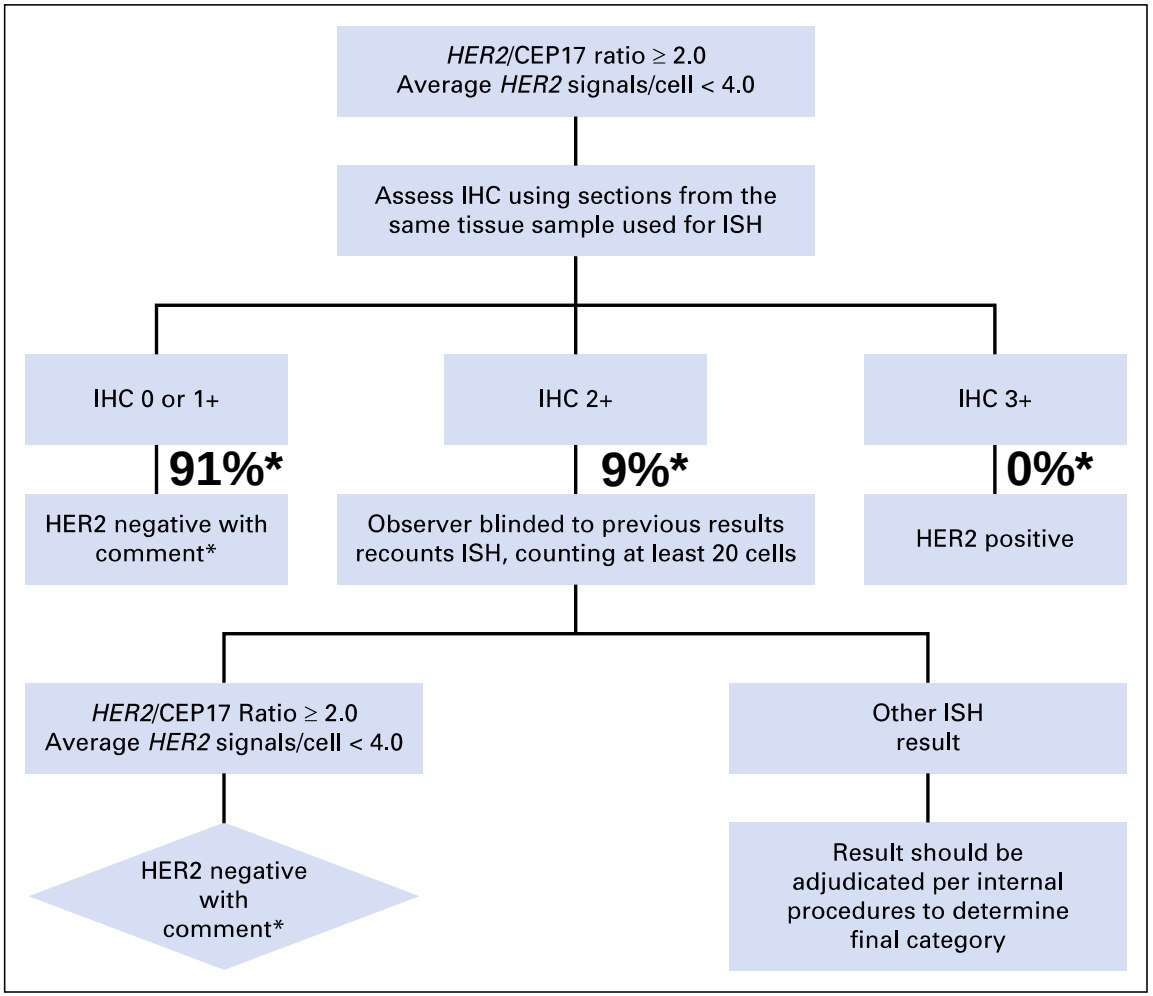


Fig 4. Clinical Question 3, group 2. (*) Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a *HER2*/CEP17 ratio ≥ 2.0 and an average *HER2* copy number of < 4.0 per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who 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.

Wolff A, et al., *JCO*, 2018

*Press MF et al., *JCO*, 2016

Comparison of *HER2* Ratio and Average *HER2* Gene Copy Number by ASCO-CAP Groupings with Clinical Outcomes in BCIRG-006 Trial

HER2 FISH Ratio	*HER2* copies per cell	No. of subjects	DFS Control (events/no. of subjects)	DFS Trastuzumab (events/ number of subjects)	DFS, HR DFS (95% CI)*	DFS, *P* for Log Rank test*	OS Control (events/no. of subjects)	OS Trastzumab	OS, HR (95% CI)*	OS *P* for Log Rank test OS*	ASCO-CAP FISH Group
Ratio ≥2.0	**<4.0**	46	4 / 18	6 / 28	1.10 (0.31, 3.89)	0.8860	2 / 18	4 / 28	3.15 (0.35, 28.63)	0.2839	**Group 2**
	≥4	3109	251 / 1031	391 / 2078	0.71 (0.60-0.83)	<.0001	138 / 1031	202 / 2078	0.69 (0.55-0.85)	0.0006	**Group 1**
Total:		3155									

NOTE. The HRs are for Trastuzumab treatment arms compared with Control chemotherapy only arm. There were too few patients (n = 5) accrued to BCRIG-006 with a HER2 FISH ratio <2.0 and ≥6.0 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.

Press et al., *Journal of Clinical Oncology*, 2016.

Evaluation of FISH Group 3

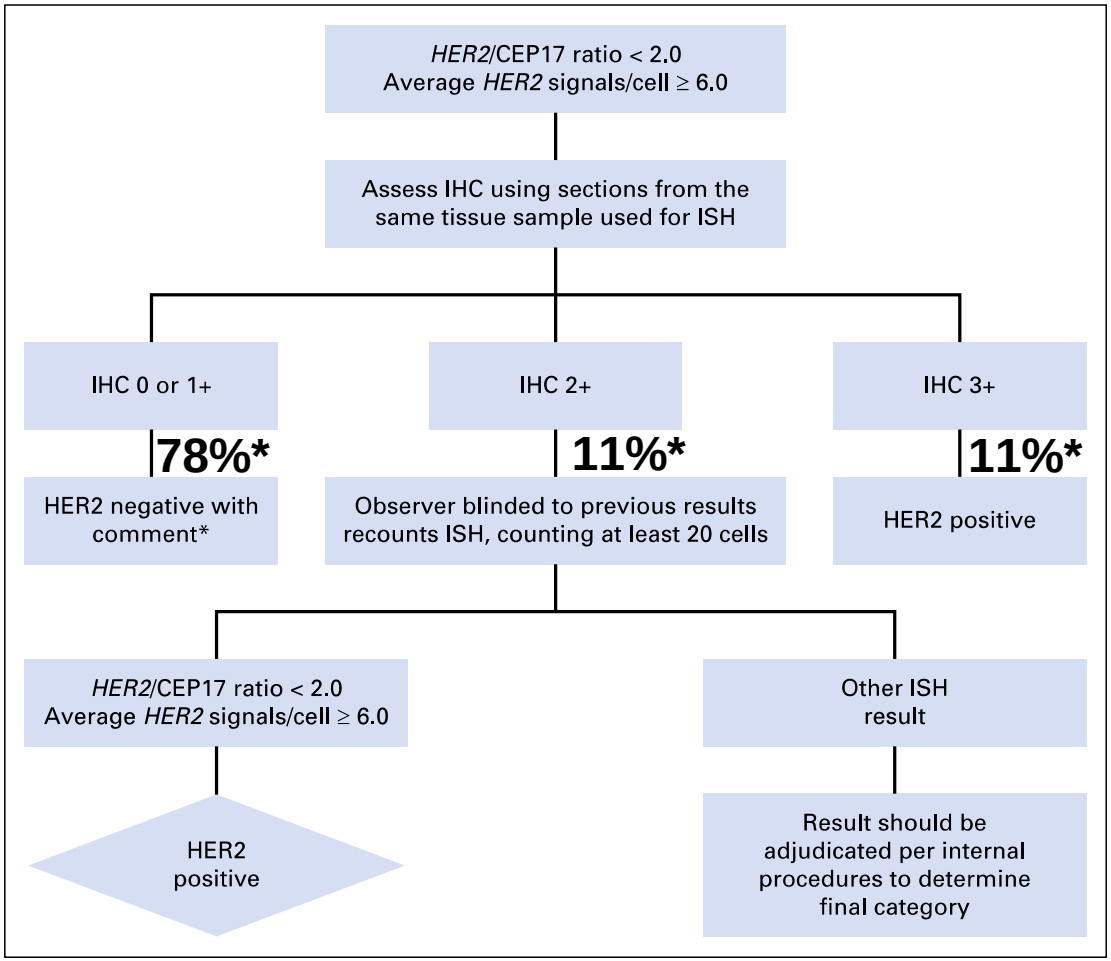


Fig 5. Clinical Question 4, group 3. (*) There are insufficient data on the efficacy of HER2-targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent 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.

Wolff A, et al., *JCO*, 2018

*Press MF et al., *JCO*, 2016

Comparison of *HER2* Gene Amplification Status with HER2 Protein Expression by a Laboratory-Developed IHC Assay (10H8-IHC) in ASCO-CAP Group 3 Patients Randomized to a BCIRG Trial.

ASCO-CAP Group (Ratio <2.0 and Average HER2 copies >6.0)	*HER2* BCIRG FISH Status	Mean of average *HER2* copy numbers	IHC 0	IHC 1+	IHC 2+	IHC 3+	Totals
Group 3A	**Amplified**	Average 16.38	1 (17%)	0 (0%)	3 (50%)	2 (33%)	6 (24%)
Group 3N	**Not Amplified**	Average 7.43	8 (42%)	9 (47%)	2 (11%)	0 (0%)	19 (76%)
			9	9	5	2	25 (100%)

There is a significant difference between Group 3A and Group 3N in terms of IHC staining with 83% of Group 3A IHC 2+/3+ compared with 89% of Group 3N that were IHC 0/1+ (p=0.002, Fisher's exact test).

Press et al., *Journal of Clinical Oncology*, 2016.

Minority of ASCO-CAP FISH Group 3 breast cancers (our "Group 3A") show *HER2* gene amplification and HER2 protein overexpression

***HER2* = 23.2 / cell**

CEP17 = 15.75 / cell

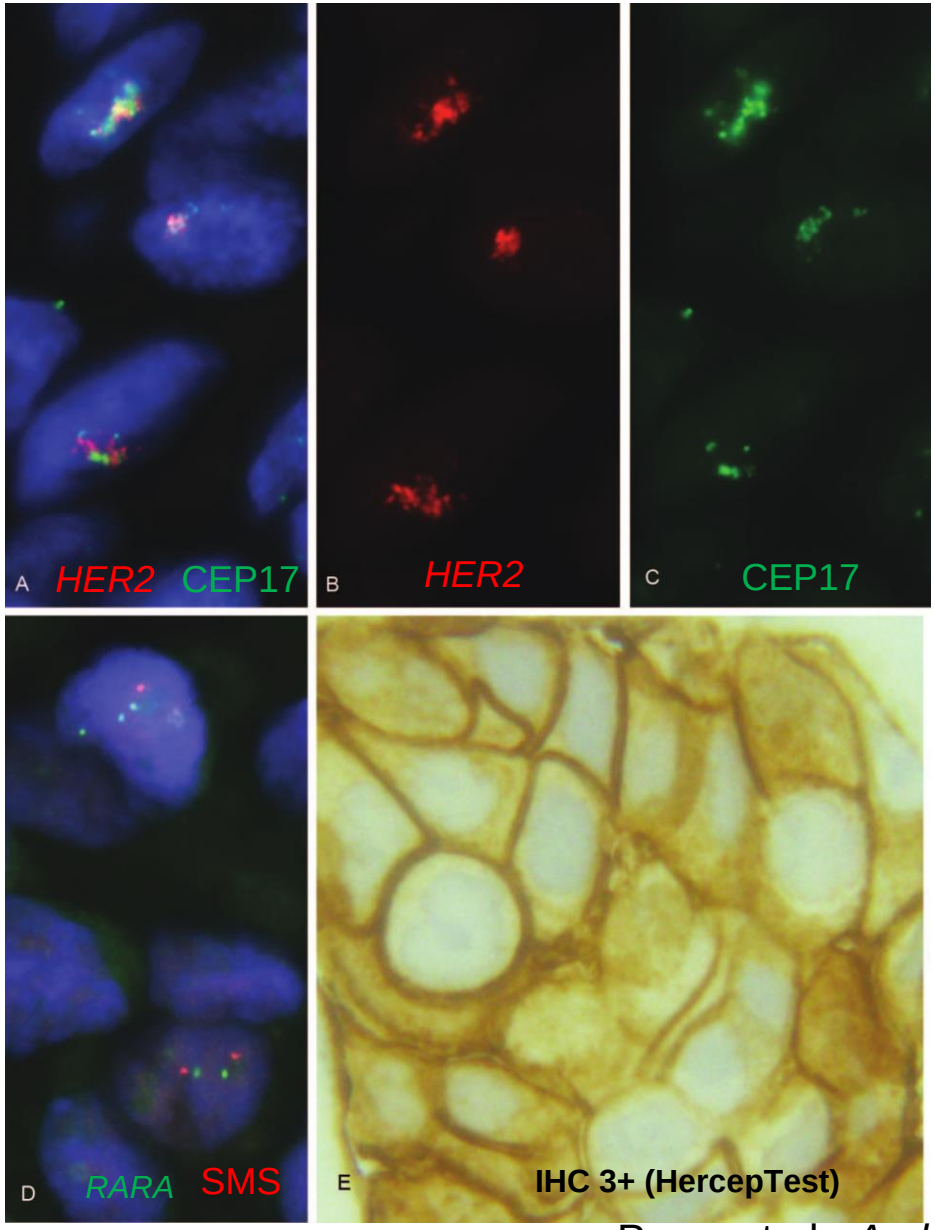
***HER2* : *CEP17* = 1.47**

***RARA* = 2.55 / cell**

***HER2* : *RARA* =**
23.2 / 2.55 = 9.1

SMS = 1.85 / cell

***HER2* : SMS =**
23.2 / 1.85 = 12.54



Press et al., *Arch Pathol Lab Med*, 2016

Evaluation of FISH Group 4

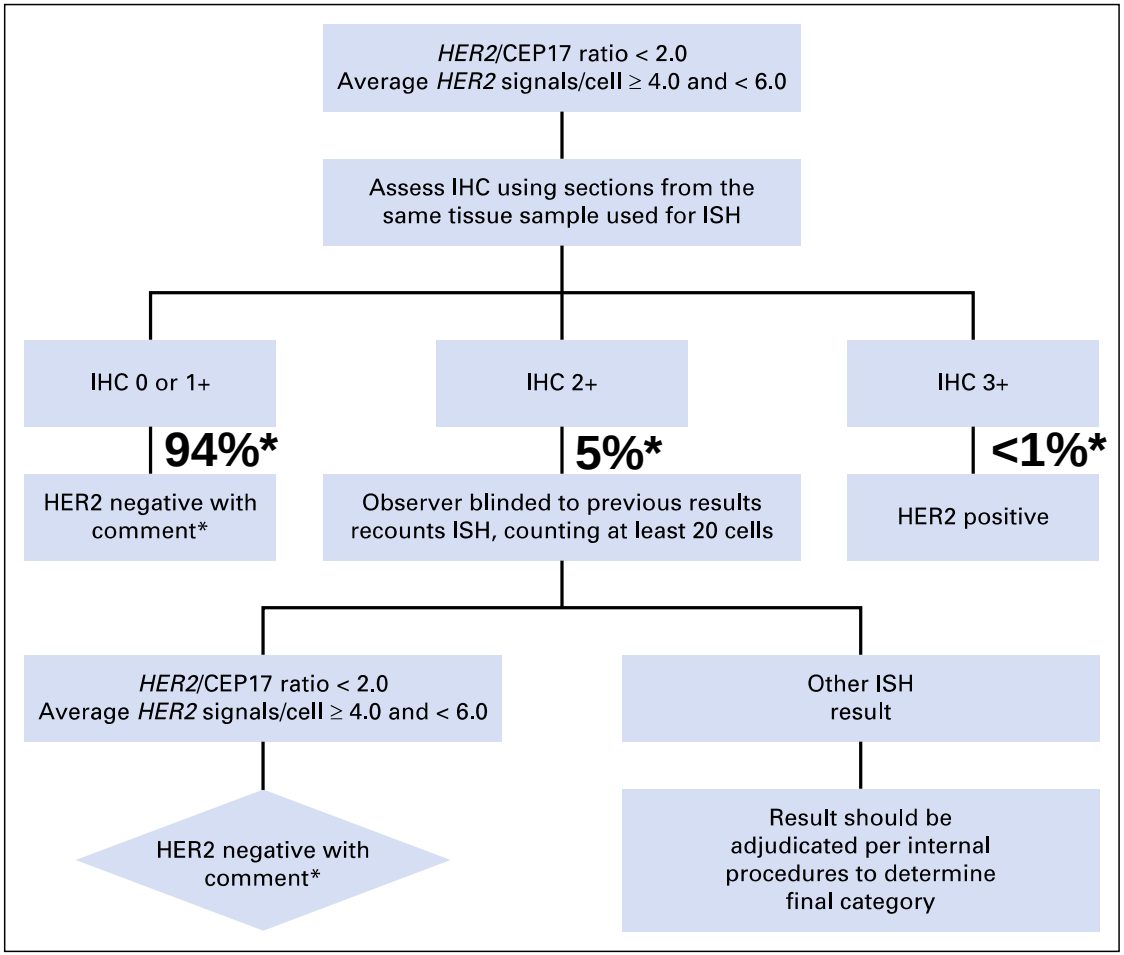


Fig 6. Clinical Question 5, group 4. (*) It is uncertain whether patients with an average of ≥ 4.0 and < 6.0 *HER2* signals per cell and a *HER2*/CEP17 ratio of < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH 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.

Wolff A, et al., *JCO*, 2018

*Press M, *JCO*, 2016

Q 6

Comparison of *HER2* Ratio and Average *HER2* Gene Copy Number by ASCO-CAP Groupings with Clinical Outcomes in BCIRG-005 Trial

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-values for logrank test*	OS, HR and P-values for logrank test*	ASCO-CAP FISH Group
Ratio <2.0	**4.01-6.0**	**176**	**51**	**30**	**0.923** (0.697-1.224) P=0.5795	**0.878** (0.609-1.267) P=0.4872	**Group 4**
Ratio <2.0	**<4.0**	**3079**	**971**	**606**	1.0 (reference)	1.0 (reference)	**Group 5**

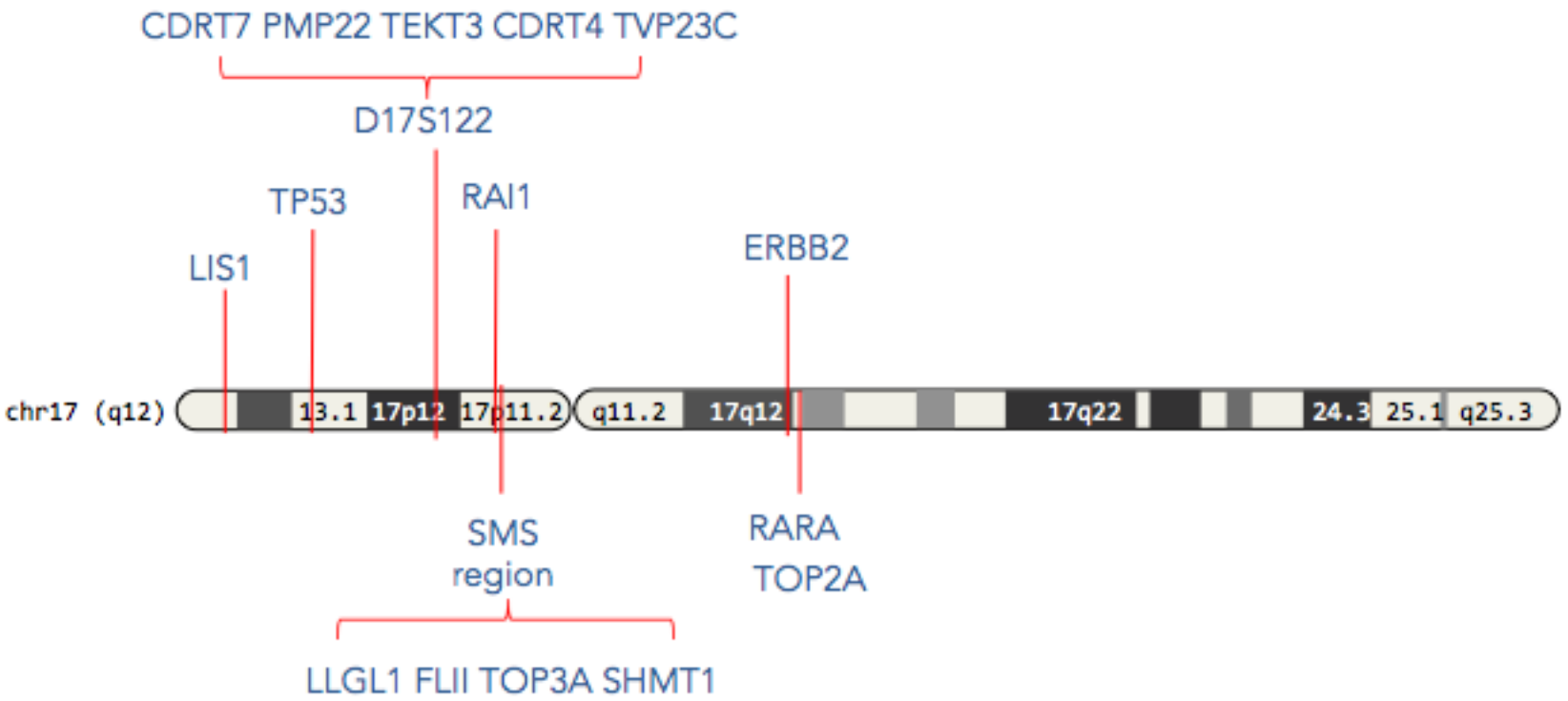
The hazard ratios are for ASCO-CAP Group 4 compared with ASCO-CAP Group 5 taken as the reference in the BCIRG-005 (*HER2*-not-amplified) breast cancer trial.

OS = overall survival
DFS = disease-free survival

Press et al., *Journal of Clinical Oncology*, 2016.

Q 6

Resolution of "*HER2* (FISH) Equivocal" Breast Cancers (ASCO-CAP Group 4) according to 2013 / 2014 ASCO-CAP Guidelines through the use of Chr 17 Alternative Control Probes



Smith-Magenis syndrome

Alternative Control Probes for *HER2* Equivocal Breast Cancers

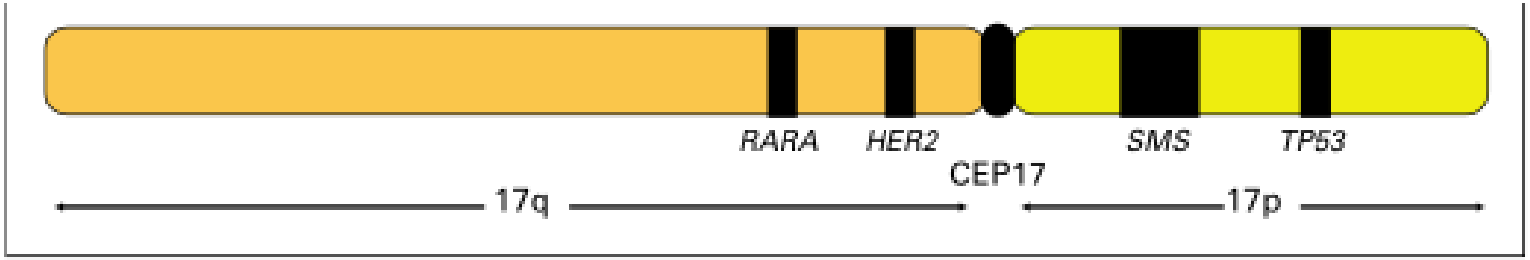
VOLUME 29 · NUMBER 31 · NOVEMBER 1 2011

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Determining True *HER2* Gene Status in Breast Cancers With Polysomy by Using Alternative Chromosome 17 Reference Genes: Implications for Anti-*HER2* Targeted Therapy

Chun Hing Tse, Harry C. Hwang, Lynn C. Goldstein, Patricia L. Kandalaft, Jesse C. Wiley, Steven J. Kussick, and Allen M. Gown



"Among the cases with mean HER2 copy number of 4 to 6, 41 (47.7%) of 86 had their HER2 gene status upgraded from nonamplified to amplified"

***HER2* copies / any Alt Control ≥2.0**

J Clin Oncol 29:4168-4174, 2011

Use of Chr 17 Alternative Control Probes for Evaluation of "*HER2* (FISH) Equivocal" Breast Cancers

Change in Pattern of *HER2* Fluorescent in Situ Hybridization (FISH) Results in Breast Cancers Submitted for FISH Testing: Experience of a Reference Laboratory Using US Food and Drug Administration Criteria and American Society of Clinical Oncology and College of American Pathologists Guidelines

Mithun Vinod Shah, Anne E. Wiktor, Reid G. Meyer, Kathleen S. Tenner, Karla V. Ballman, Stefan J. Green, William R. Sukov, Rhett P. Ketterling, Edith A. Perez, and Robert B. Jenkins

Mayo Clinic:
JCO, 34: 3502-3510, 2016

Of 405 patients initially considered FISH-equivocal (ratio <2.0 with HER2 signal ≥4.0, but <6.0, use of an alternative chromosome 17 probe reassigned 212 patients to FISH-positive: (52.3%).

Prognostic Significance of Equivocal Human Epidermal Growth Factor Receptor 2 Results and Clinical Utility of Alternative Chromosome 17 Genes in Patients With Invasive Breast Cancer: A Cohort Study

Nour Sneige, MD[1]; Kenneth R. Hess, PhD[2]; Asha S. Multani, PhD[3]; Yun Gong, MD[1]; and Nuhad K. Ibrahim, MD[4]

M. D. Anderson Cancer Ctr:
Cancer, 123: 1115-1123, 2017.

57 *HER2* "equivocal" to 35 "amplified" with D17S122: 61%

Impact of an Alternative Chromosome 17 Probe and the 2013 American Society of Clinical Oncology and College of American Pathologists Guidelines on Fluorescence in Situ Hybridization for the Determination of *HER2* Gene Amplification in Breast Cancer

Alana R. Donaldson, MD[1]; Shashirekha Shetty, PhD[2]; Zhen Wang, MD, PhD[1]; Christine L. Rivera, BS[2]; Bryce P. Portier, MD, PhD[1]; G. Thomas Budd, MD[3]; Erinn Downs-Kelly, DO[4]; Christopher P. Lanigan, MS[1]; and Benjamin C. Calhoun, MD, PhD[1]

Cleveland Clinic:
Cancer 123: 2230-2239, 2017.

73 *HER2* "equivocal" to 38 "amplified" with D17S122: 52%

TABLE 3. Clinical and Pathologic Characteristics of Patients Reclassified From Equivocal to Amplified Using the *D17S122* Probe and the 2013 American Society of Clinical Oncology/College of American Pathologists Guidelines (n = 38)

	CEP17 Probe Set, Average Copy Numbers and Ratio				*D17S122* Probe Set, Average Copy Numbers and Ratio									
Patient	*HER2*	Chr17	Ratio	*HER2* Status	*HER2*	Chr17	Ratio	HER2 Status	Anti-HER2 Therapy	HER2 IHC	ER, %	PR, %	Grade	Pos. LN
1	4.6	3.2	1.4	Equivocal	3.9	1.6	2.4	Amplified	Yes	NA	90	90	3	0
2	4.4	3.3	1.3	Equivocal	5.1	2.1	2.4	Amplified	No	0	90	70	2	1
3	4.6	3.5	1.3	Equivocal	4.6	1.7	2.7	Amplified	Yes	NA	95	95	2	3
4	5.1	3.9	1.3	Equivocal	5.4	1.9	2.8	Amplified	Yes	NA	90	90	2	0
5	4.3	3.2	1.3	Equivocal	4.8	1.9	2.5	Amplified	Yes	NA	95	5	3	1
6	4.3	3.6	1.2	Equivocal	4.0	1.9	2.1	Amplified	Yes	2+	60	50	3	1
7	5.7	3.2	1.8	Equivocal	7.0	2.5	2.8	Amplified	Yes	NA	0	0	3	0
8	4.3	3.1	1.4	Equivocal	4.0	1.9	2.1	Amplified	No	2+	80	90	1	0
9	4.9	4.2	1.2	Equivocal	5.6	2.0	2.8	Amplified	Yes	NA	95	60	2	0
10	4.3	2.8	1.5	Equivocal	4.6	1.7	2.7	Amplified	No	NA	90	2	3	0
11	5.3	4.2	1.3	Equivocal	7.1	2.6	2.7	Amplified	Yes	NA	90	90	3	2
12	4.5	3.9	1.2	Equivocal	6.2	1.7	3.6	Amplified	Yes	NA	95	95	2	2
13	4.8	3.7	1.3	Equivocal	3.6	1.8	2.0	Amplified	Yes	2+	100	100	3	0
14	5.1	2.7	1.8	Equivocal	5.6	1.5	3.7	Amplified	No	2+	95	90	3	0
15	4.5	3.8	1.2	Equivocal	4.7	2.0	2.4	Amplified	Yes	NA	95	70	2	3
16	4.1	3.1	1.3	Equivocal	5.4	1.9	2.8	Amplified	Yes	NA	95	80	2	0
17	4.6	3.3	1.4	Equivocal	4.5	2.0	2.3	Amplified	Yes	2+	99	85	2	0
18	5.6	3.1	1.8	Equivocal	5.2	1.8	2.9	Amplified	Yes	NA	95	40	2	0
19	4.3	3.0	1.4	Equivocal	4.7	2.1	2.2	Amplified	Yes	2+	100	40	3	0
20	4.9	3.3	1.5	Equivocal	4.6	1.7	2.7	Amplified	Yes	NA	100	90	2	NA
21	4.8	3.5	1.4	Equivocal	5.9	1.8	3.3	Amplified	No	NA	95	30	3	0
22	5.0	4.0	1.3	Equivocal	6.0	2.1	2.9	Amplified	Yes	NA	90	5	3	5
23	4.4	3.6	1.2	Equivocal	5.1	2.0	2.6	Amplified	Yes	2+	100	80	3	0
24	4.2	3.2	1.3	Equivocal	4.7	1.8	2.6	Amplified	Yes	NA	80	90	2	0
25	4.2	3.2	1.3	Equivocal	5.4	1.8	3.0	Amplified	Yes	NA	95	10	3	0
26	4.3	3.6	1.2	Equivocal	4.9	2.0	2.4	Amplified	No	NA	95	20	2	0
27	5.1	3.7	1.4	Equivocal	6.2	3.8	1.6	Amplified	Yes	3+	0	0	3	0
28	4.5	3.3	1.4	Equivocal	8.0	1.8	4.4	Amplified	Yes	NA	NA	NA	2	NA
29	5.0	3.2	1.6	Equivocal	5.4	1.8	3.0	Amplified	NA	NA	95	95	2	0
30	4.9	3.3	1.5	Equivocal	5.5	1.8	3.1	Amplified	NA	2+	90	70	3	0
31	4.0	3.1	1.3	Equivocal	3.5	1.6	2.2	Amplified	Yes	1+	NA	NA	3	NA
32	4.2	3.6	1.2	Equivocal	3.6	1.1	3.3	Amplified	Yes	2+	100	5	2	NA
33	4.4	3.3	1.3	Equivocal	4.3	1.8	2.4	Amplified	No	2+	50	0	2	16
34	4.9	3.7	1.3	Equivocal	4.4	1.6	2.8	Amplified	NA	NA	NA	NA	NA	NA
35	4.3	2.9	1.5	Equivocal	4.7	1.6	2.9	Amplified	NA	NA	95	95	2	NA
36	5.6	3.5	1.6	Equivocal	7.3	1.7	4.3	Amplified	NA	NA	90	0	3	NA
37	5.1	2.7	1.9	Equivocal	5.2	1.2	4.3	Amplified	NA	NA	95	80	3	0
38	4.3	3.0	1.4	Equivocal	4.2	1.8	2.3	Amplified	NA	NA	0	0	3	NA

38 of 73 (52%) "*HER2* equivocal" breast cancers were "re-classified" as "amplified"
(Donaldson AR, et al. *Cancer*, 2017)

Importance of an Appropriate Internal Control for Assessment of Amplification

Studies of the HER-2/*neu* Proto-oncogene in Human Breast and Ovarian Cancer

DENNIS J. SLAMON,* WILLIAM GODOLPHIN, LOVELL A. JONES, JOHN A. HOLT, STEVEN G. WONG, DUANE E. KEITH, WENDY J. LEVIN, SUSAN G. STUART, JUDY UDOVE, AXEL ULLRICH, MICHAEL F. PRESS

"We evaluated 345 patients with node positive disease in a blinded fashion (Table 1). Of these, 101 (27%) had evidence of HER-2/neu amplification. Univariate (as well as multivariate) survival analysis showed amplification of the HER-2/neu gene to be a significant predictor of both disease-free survival and overall survival for these patients (Table 1)." (Slamon et al., *Science* 244: 707-712, 1989) NOTE: *MPO* was the internal control gene for assessment of amplification, i.e. a *HER2*-to-*MPO* ratio ≥2.0.

[CANCER RESEARCH 51, 944–948, February 1, 1991]

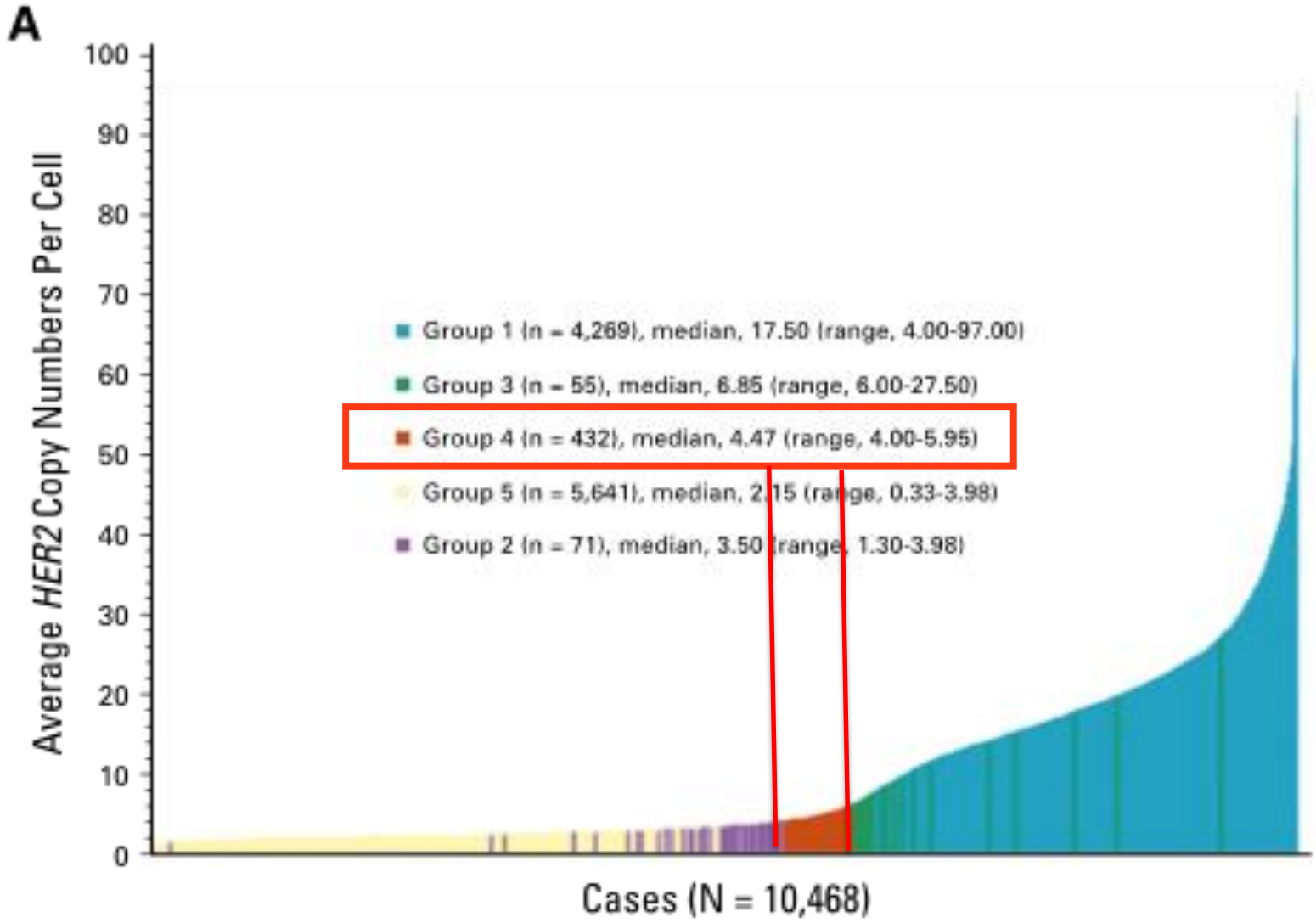
Follow-up Study of HER-2/*neu* Amplification in Primary Breast Cancer[1]

Gary M. Clark[2] and William L. McGuire

Department of Medicine/Medical Oncology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284–7884

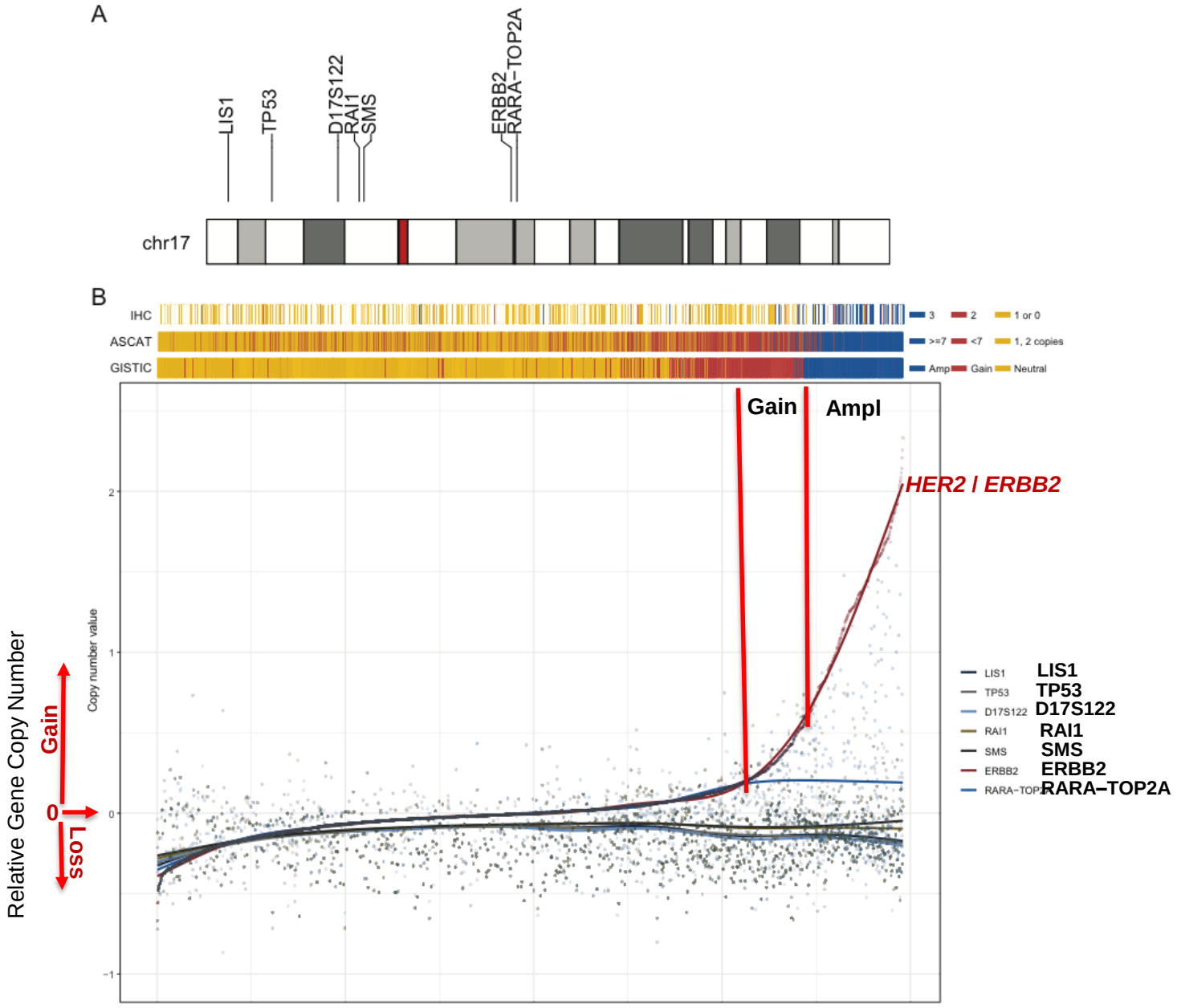
"HER-2/neu amplification was determined by the ratio of the HER-2/neu signal relative to the single copy p53 signal." "The overall amplification rate was 33%." "Amplification of the HER-2/neu gene did not correlate with either disease-free or overall survival in univariate or multivariate analyses." (Clark and McGuire, *Cancer Res*. 51, 944-948, 1991)

Distribution of average HER2 gene copies and HER2 FISH ratios among breast cancers successfully screened for enrollment into BCIRG trials from 2000 to 2004



Press et al., *JCO*, 2016

Relative Copy Number of *HER2 / ERBB2* and Genomic Sites used as Alternative Controls to determine HER2 Status by FISH (METABRIC SNP array data; N = 1980)



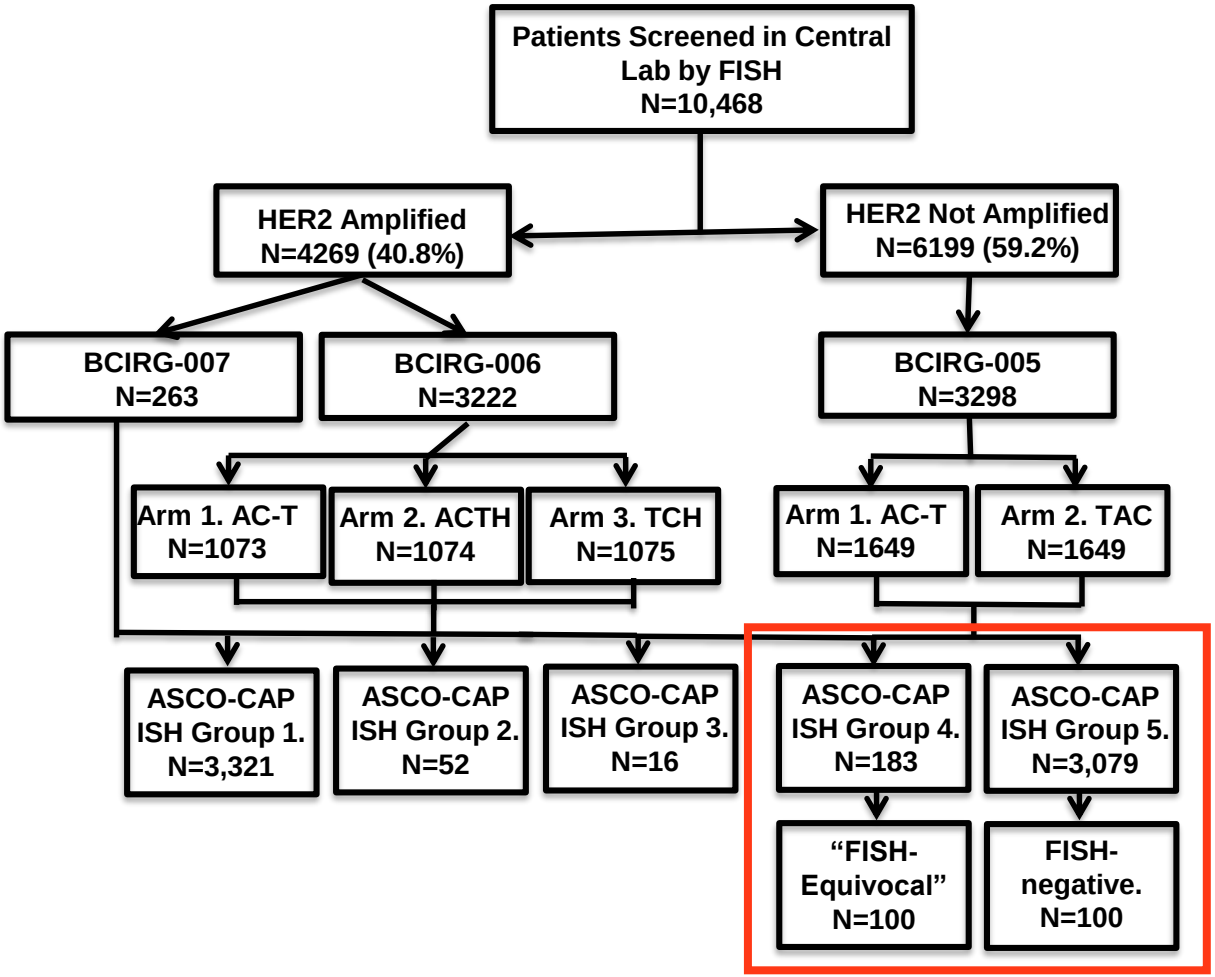
Press MF, Seoane JA, Curtis C et al. *JAMA Oncology*. 2019

Chromosome 17 Regional Gene Copy Gains / Losses based on GISTIC among Alternative Control Genomic Sites Compared to *HER2/ERBB2* Gene Copy Gains / Losses in the METABRIC Cohort (N = 1915)

	HER2 Copy Number Status									
Alternative Control (Region)	**HER2 Loss**		**HER2 Normal**		**HER2 Gain**		**HER2 Amp**		**Total**	
LIS1										
Gain	9	2.48%	25	2.67%	61	18.71%	13	4.47%	108	5.64%
Normal	46	12.67%	622	66.52%	64	19.63%	87	29.90%	819	42.77%
Loss	308	84.85%	288	30.80%	201	61.66%	191	65.64%	988	51.59%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%
TP53										
Gain	3	0.83%	15	1.60%	60	18.40%	4	1.37%	82	4.28%
Normal	41	11.29%	624	66.74%	59	18.10%	81	27.84%	805	42.04%
Loss	319	87.88%	296	31.66%	207	63.50%	206	70.79%	1028	53.68%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%
D17S122 (TEKT3)										
Gain	10	2.75%	11	1.18%	55	16.87%	8	2.75%	84	4.39%
Normal	50	13.77%	643	68.77%	67	20.55%	84	28.87%	844	44.07%
Loss	303	83.47%	281	30.05%	204	62.58%	199	68.38%	987	51.54%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%
RAI1										
Gain	7	1.93%	39	4.17%	78	23.93%	35	12.03%	159	8.30%
Normal	57	15.70%	640	68.45%	64	19.94%	89	30.58%	851	44.44%
Loss	299	82.37%	256	27.38%	183	56.13%	167	57.39%	905	47.26%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%
SMS (TOP3A)										
Gain	12	3.31%	42	4.49%	79	24.23%	41	14.09%	174	9.09%
Normal	54	14.88%	647	69.20%	71	21.78%	94	32.30%	866	45.22%
Loss	297	81.82%	246	26.31%	176	53.99%	156	53.61%	875	45.69%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%
TOP2A/RARA (TOP2A)										
Gain	2	0.55%	19	2.03%	284	87.12%	117	40.21%	422	22.04%
Normal	22	6.06%	899	96.15%	36	11.04%	77	26.46%	1034	53.99%
Loss	339	93.39%	17	1.82%	6	1.84%	97	33.33%	459	23.97%
Total	363	100%	935	100%	326	100%	291	100%	1915	100%

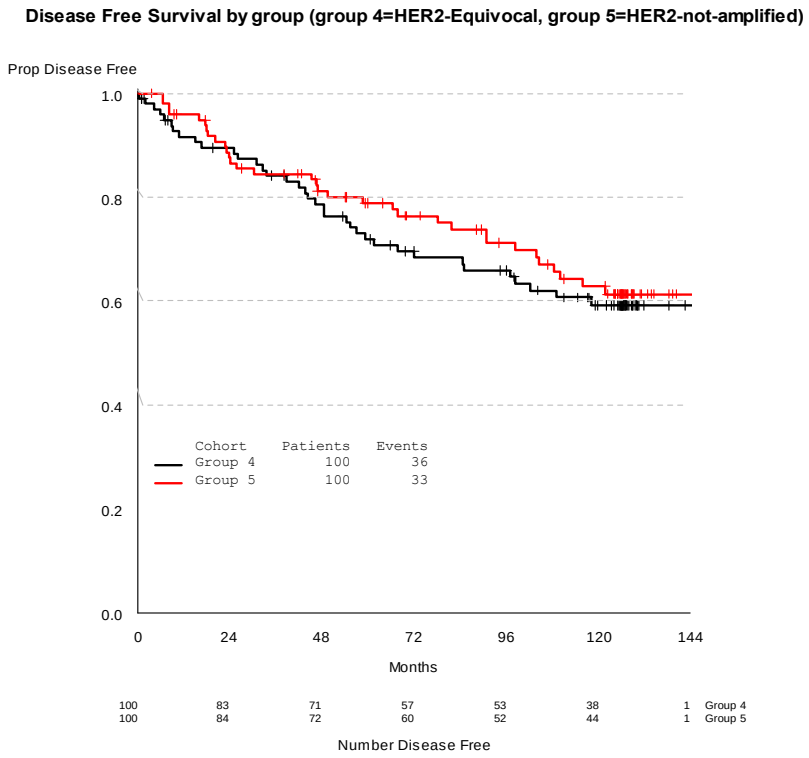
Press MF, Seoane JA, Curtis C et al. *JAMA Oncology*, 2019

Evaluation of *HER2*-Equivocal and *HER2*-Not-Amplified Breast Cancers by FISH: Specimen Accountability

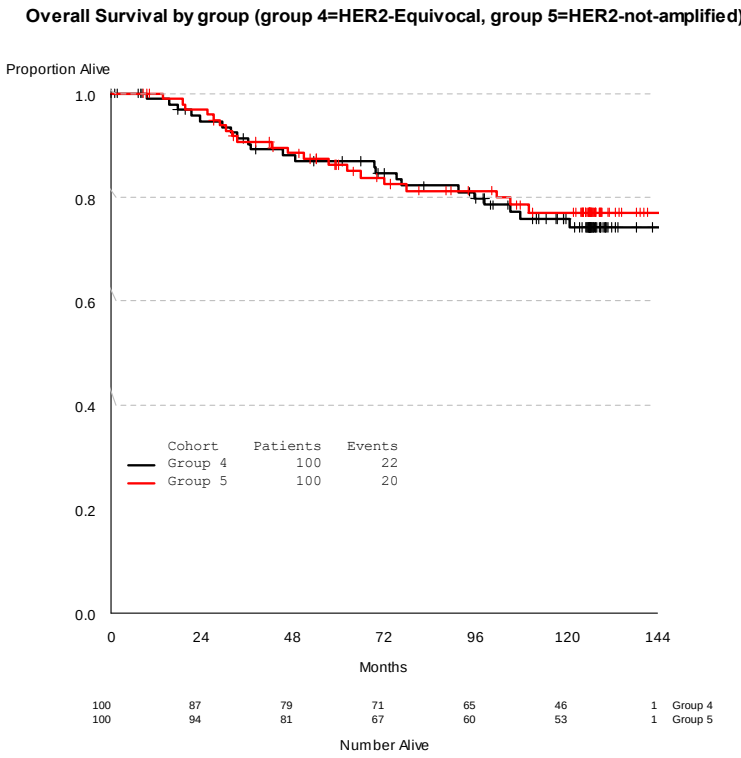


JAMA Oncology, 2019

Outcomes for ASCO-CAP Group 4 (*HER2*-Equivocal) and ASCO-CAP Group 5 (*HER2*-not-amplified) Breast Cancer Patients: DFS and OS.



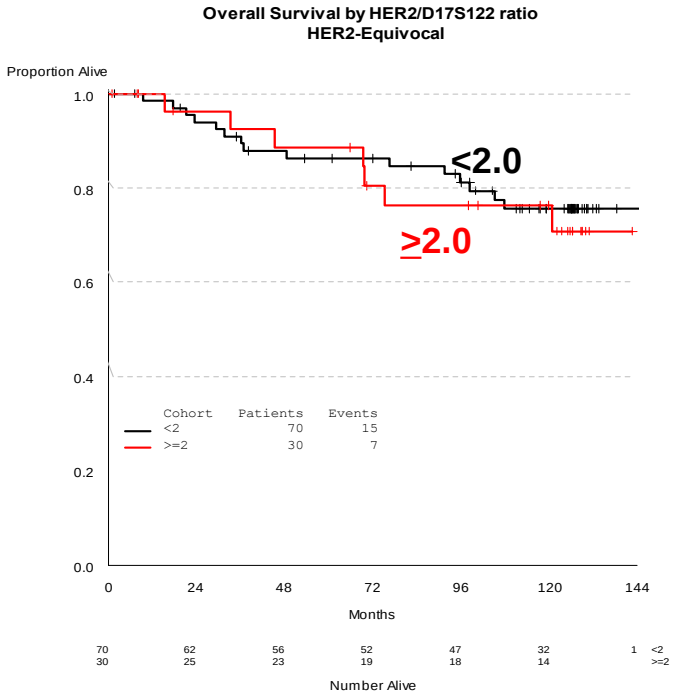
Disease-Free Survival of **ASCO-CAP FISH Group 4 (HER2-Equivocal)** Compared to **ASCO-CAP FISH Group 5 (HER2-negative)**

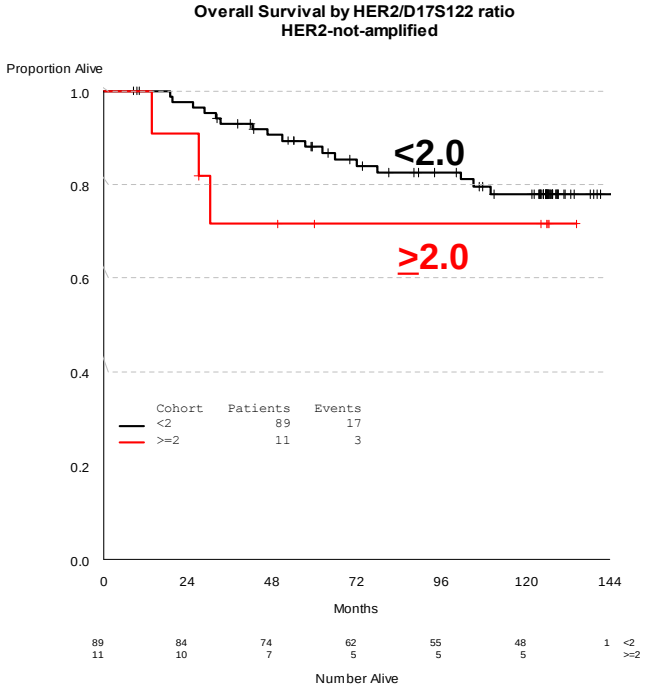


Overall Survival of **ASCO-CAP FISH Group 4 (HER2-Equivocal)** Compared to **ASCO-CAP FISH Group 5 (HER2-negative)**

JAMA Oncology, 2019

Overall Survival for ASCO-CAP Group 4 (*HER2*-Equivocal) and ASCO-CAP Group 5 (*HER2*-not-amplified) Breast Cancer Patients by HER2 / Alternative Probe Ratios





ASCO-CAP FISH Group 4 (HER2-**Equivocal**): OS for **HER2 / D17S122 Ratios ≥2.0** versus **Ratios <2.0**

ASCO-CAP FISH Group 5 (HER2-**negative**): OS for **HER2 / D17S122 Ratios ≥2.0** versus **Ratios <2.0**

JAMA Oncology, 2019

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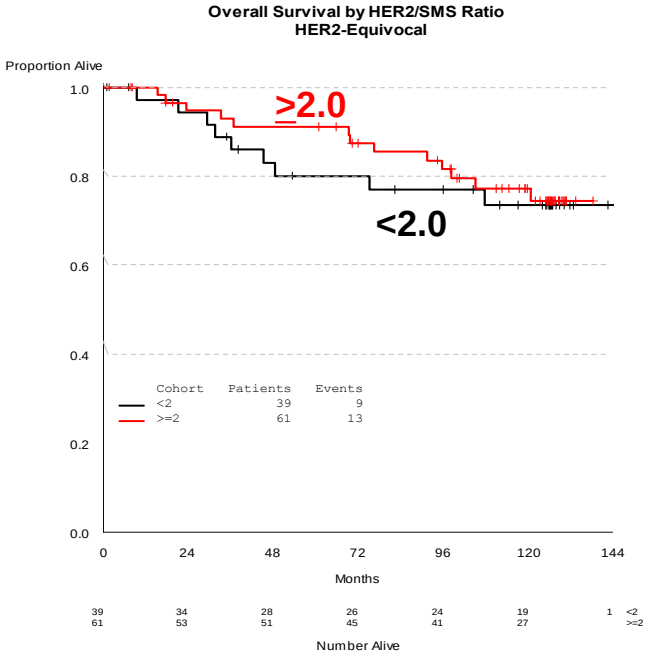
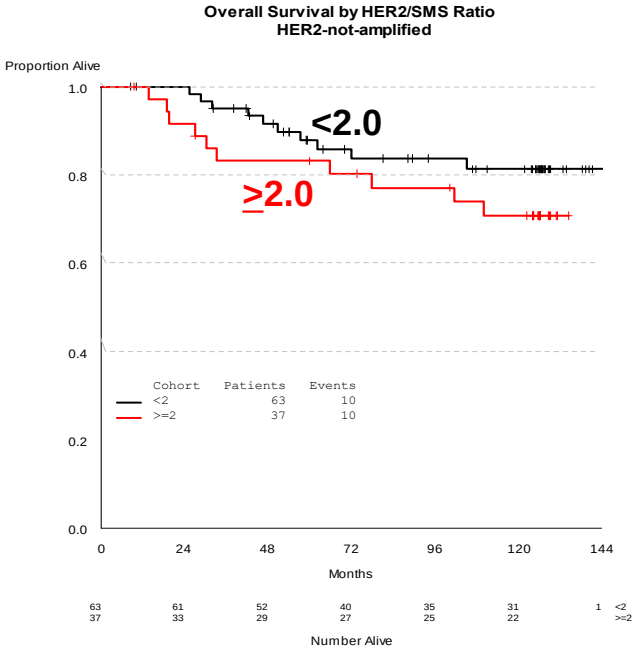
Overall Survival for ASCO-CAP Group 4 (*HER2*-Equivocal) and ASCO-CAP Group 5 (*HER2*-not-amplified) Breast Cancer Patients by HER2 / Alternative Probe Ratios





ASCO-CAP FISH Group 4 (HER2-**Equivocal**): OS for **HER2 / SMS Ratios ≥2.0** versus **Ratios <2.0**

ASCO-CAP FISH Group 5 (HER2-**negative**): OS for **HER2 / SMS Ratios ≥2.0** versus **Ratios <2.0**

JAMA Oncology, 2019

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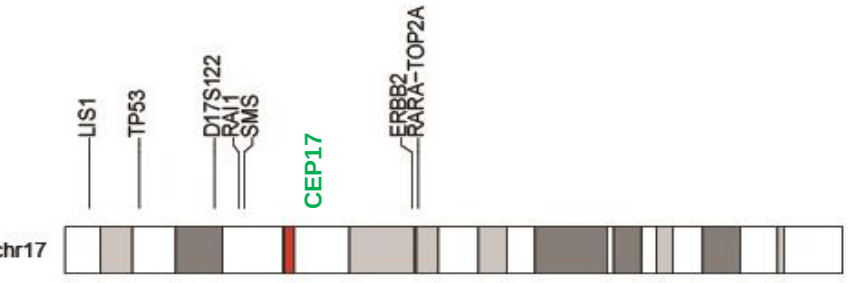
Criteria for Evaluation of Heterozygous Deletions at Alternative Control Genomic Sites on Chromosome 17 by FISH

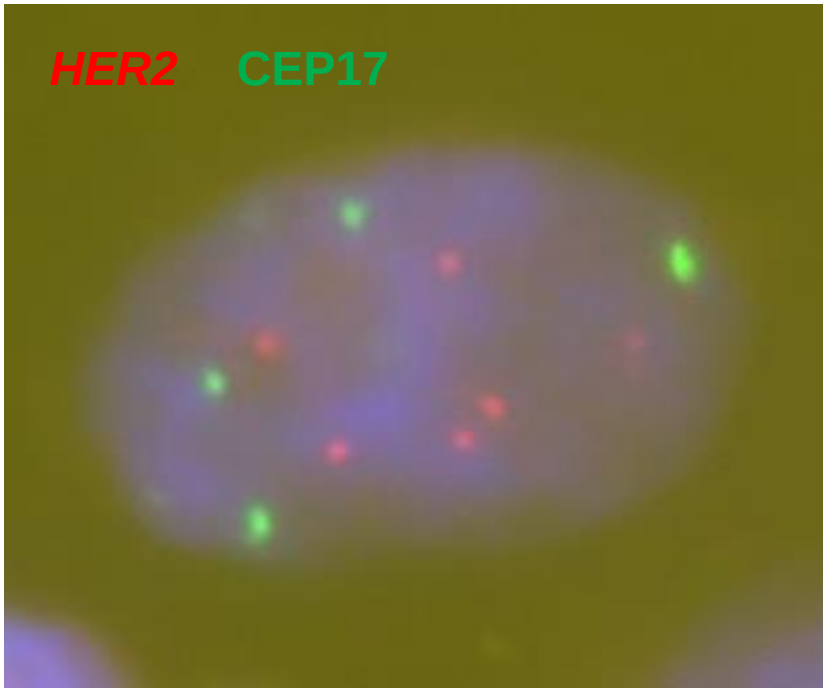
Chromosome 17 Arm	Gene / Locus	Ratio	Interpretation	Ratio	Interpretation
p-arm	SMS	<0.75[a]	SMS with heterozygous deletion relative to *RARA*	>1.25[d]	*RARA* with heterozygous deletion relative to SMS
q-arm	*RARA*				
p-arm	*TP53*	<0.75[b]	*TP53* with heterozygous deletion relative to *TOP2A*	>1.25[e]	*TOP2A* with heterozygous deletion relative to *TP53*
q-arm	*TOP2A*				
p-arm	D17S122	<0.75[c]	D17S122 with heterozygous deletion relative to *HER2*	>1.25[f]	*HER2* with heterozygous deletion relative to D17S122
q-arm	*HER2*				

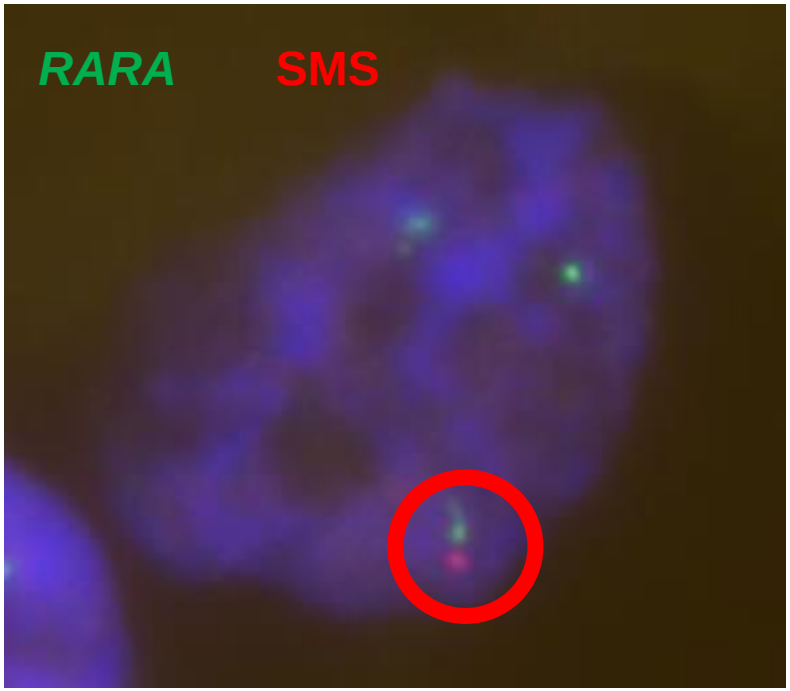


SMS, Smith-Magenis syndrome locus; *RARA*, retinoic acid receptor-alpha gene; *TP53*, tumor protein 53 tumor suppressor gene; *TOP2A*, topoisomerase-II-alpha gene; D17S122: the 17p-arm genomic locus which is duplicated in Charcot-Marie-Tooth disease; *HER2*, human epidermal growth factor receptor 2 gene.

JAMA Oncology, 2019

HER2 Equivocal by FISH: Heterozygous Deletion of SMS relative to *RARA*





***HER2* / CEP17 = 4.42 / 2.58 = 1.72**

***HER2* / *RARA* = 4.42 / 4.40 = 1.00**

***HER2* / SMS = 4.42 / 2.05 = 2.15**

HER2 IHC by 10H8 assay: IHC 0
HER2 IHC by Dako HercepTest: IHC 1+

Press et al. *JAMA Oncology*, 2019

Comparison of FISH Groups with FDA-Approved Status, ASCO-CAP Guidelines Recommendations, HER2 Protein Expression by IHC, and Associations with Outcomes in BCIRG Clinical Trials

Grp	Ratio	Average *HER2*	%	FDA	ASCO-CAP 2014 2018	HER2 Protein	Progn BCRIG-005	Trast Resp BCIRG-006	BCIRG / TRIO
1	≥ 2.0	≥ 4.0	40.8	Ampl	ISH +	Overexp	-	Signific Improv	Amplified
2	≥ 2.0	< 4.0	0.7	Ampl	ISH+ IHC	Low Ex	-	Not sig	Not Ampl
3	< 2.0	≥ 6.0	0.5	Not Am	ISH+ IHC	Mixed	Indeter	Indeter	Mixed
4	< 2.0	≥ 4.0, <6.0	4.1	Not Am	ISH? IHC	Low Ex	Not Worse	-	Not Ampl
5	< 2.0	< 4.0	53.9	Not Am	ISH -	Low Ex	Reference	-	Not Ampl

Press et al., *Journal of Clinical Oncology*, 2016; *JAMA Oncology*, 2019.

Conclusions

- Development of Companion Diagnostics for clinical trials and patient management are complex regulatory as well as research issues.
- In spite of three decades of research, HER2 testing for selection of patients to targeted therapies remains controversial.
  - Implementation of new ASCO-CAP guidelines for *HER2* FISH testing result in no changes for approximately 90%-95% of cases.
  - Changes in ASCO-CAP guidelines for “groups 2-4” will result in potential disagreements for approximately 5% of cases.
- The use of Chr 17 alternative control genes, especially p-arm genes, as alternative controls to assess *HER2* gene status may lead to “false-positive” ratios by FISH due to heterozygous deletion of chromosome 17p-arm genomic sites.

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