

**PROBLEMATICHE INERENTI LA  
DETERMINAZIONE DELLO STATO DI HER 2**

**IMMUNOISTOCHEMICA: PIETRIBIASI  
ISH: MARCHIO'**

# ICC

- PREANALITICA
- PROCESSAZIONE
- VALUTAZIONE
- CORRELAZIONE CON ALTRI PARAMETRI
- C.D.Q

COLORAZIONE OTTIMALE

## **FASE PRE-ANALITICA**

inizio, tipo e durata della fissazione

# **PROCESSAZIONE**

Coloratore, ab, smascheramento ag

# **FASE ANALITICA**

# ICC

- **DIFFICILE**
- **SOGGETTIVA**
- **“COMPLETA”, “INCOMPLETA” “INTENSA” “ MODERATA”**
- **% DI CELLULE**
- **MANCANZA CONTROLLO INTERNO**

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A S C O S P E C I A L A R T I C L E

VOLUME 31 · NUMBER 31 · NOVEMBER 2013

JOURNAL OF CLINICAL ONCOLOGY

A S C O S P E C I A L A R T I C L E

## 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\**

# CONFRONTO TRA LINEE GUIDA ASCO CAP 2007 E 2013

## Limiti delle linee guida 2007 e obiettivi di miglioramento

Le linee guida ASCO CAP pubblicate nel 2007 necessitavano da tempo di una revisione e dell'aggiornamento di alcuni aspetti, come auspicato da più parti nella comunità scientifica.

Uno degli obiettivi da perseguire era la riduzione dei risultati falsamente positivi o negativi al di sotto del 5% complessivamente, tramite indicazioni su come gestire i casi per i quali i risultati di laboratorio divergono dall'atteso o non sono coerenti nella stessa paziente.

In letteratura le quote di false positività e di false negatività in IHC sono circa del 15-20% e del 10% rispettivamente, le quote di false negatività in FISH sono del 10-15%.

o del 10% (criteri FDA) può essere decisiva per l'accesso al trattamento con trastuzumab di 3.000-5.000 donne. **La conseguenza possibile in caso di falsa negatività è infatti negare a una paziente l'accesso a una terapia appropriata con trastuzumab.**



## HER2 positivity rate

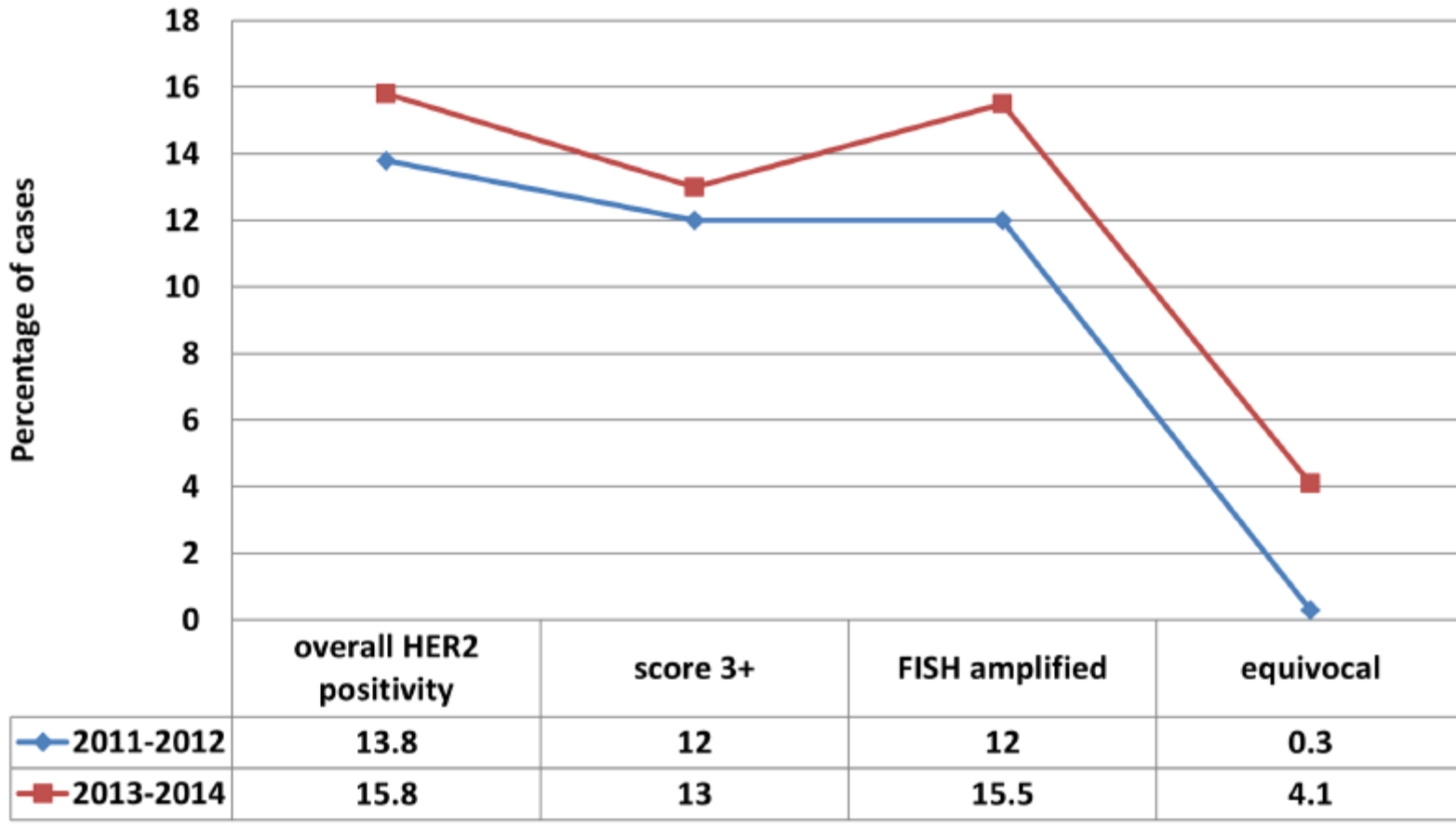
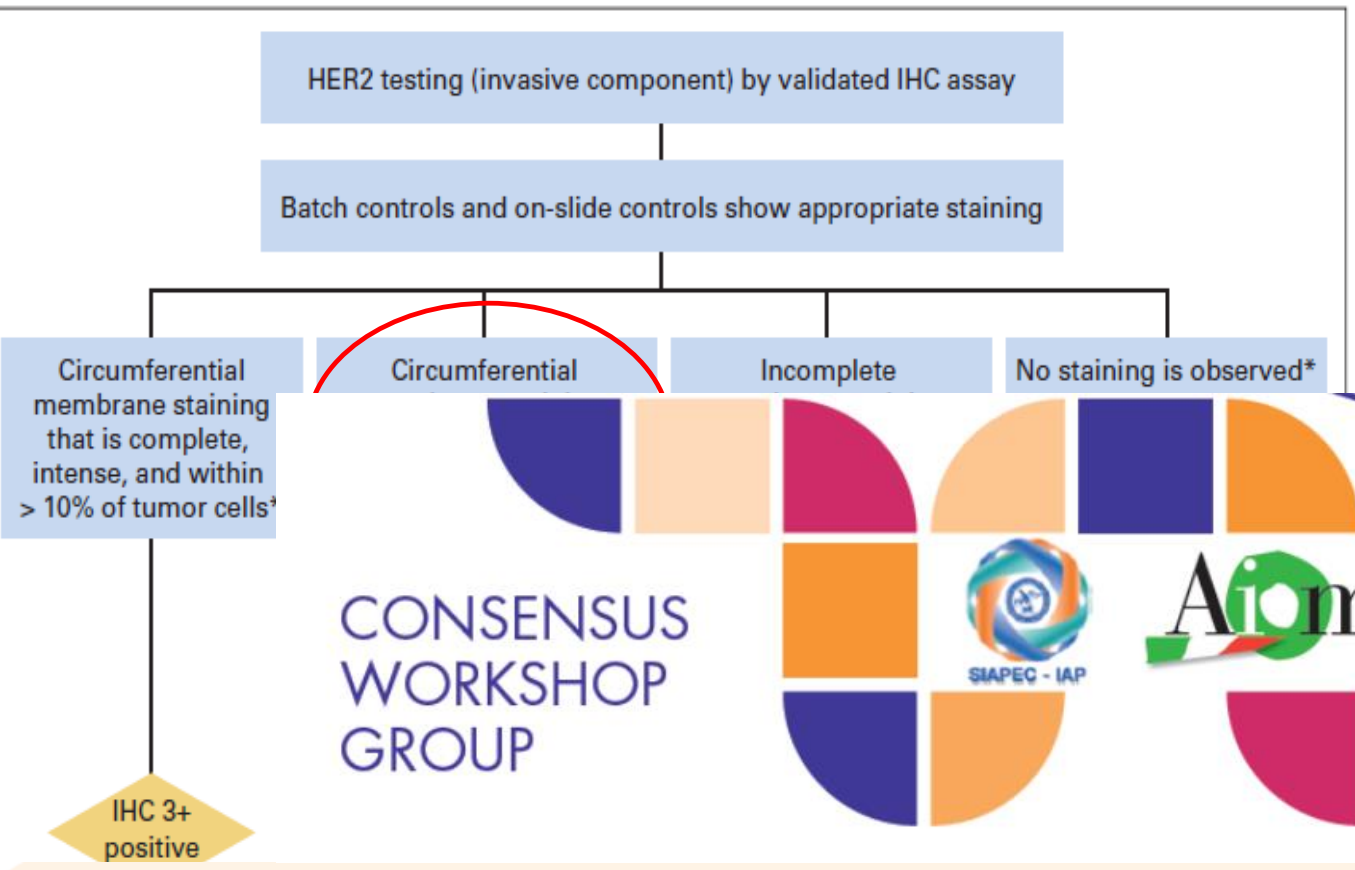


Fig 1. Diagrammatic representation of HER2 positivity rate in percentage in the periods of 2011–2012 (two years) and from October 2013 to October 2014 (12 months).



**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. The categories of HER2 status by IHC can be created that are not covered by the definitions, in practice they are not used if encountered should be considered equivocal. ISH, in situ hybridization. NOTE: the final reported results should indicate that there is no apparent histologic discordance observed by the pathologist. (\*) Readily appreciated using low magnification, objective and observed in a homogeneous and contiguous cell population.

La definizione di **equivoco** può essere chiarita come segue:

- colorazione completa debole-moderata, circonferenziale in >10% delle cellule (ex 2+)
  - colorazione incompleta (baso-laterale o laterale) moderata o intensa in >10% delle cellule (ex 1+)
  - colorazione completa intensa circonferenziale in <10% delle cellule (ex 0).
- **1+ o negativo** colorazione incompleta debole o debolissima in >10% delle cellule
  - **0 o negativo** assenza di colorazione o colorazione incompleta debole o debolissima in ≤10% delle cellule.

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

for positivity or tumor heterogeneity consistent with others.<sup>3,9,10</sup> Furthermore, we have reviewed 3,054 breast cancer tissue samples consecutively reported in routine practice in the United States in the last 4 years (2010 to 2013). Ninety-eight cases (3%) had HER2 status assessed on core biopsy and the corresponding tumor excision specimen. Of those 98, the HER2 status of the index tumor was changed in two, and both were in the borderline result category (ratio, 1.8 to 2.2<sup>1</sup>). Importantly, no contribution of histological grade, tumor type, or hormone receptor status was identified.

If retesting on excision is mandated to pick up such a small number of discrepant cases that mainly have borderline low-level HER2 amplification status, the same argument can be applied to repeating the test on all tumor tissue blocks from the excision speci-

## Reply to E.A. Rakha et al

We thank Rakha et al<sup>1</sup> for their correspondence concerning the 2013 American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) human epidermal growth factor receptor 2 (HER2) testing guideline update for invasive breast cancer.<sup>2,3</sup> We also appreciate their previous thoughtful commentary and concur that the recommendations of the ASCO/CAP Panel were aimed to “improve the analytic validity of HER2 testing, its clinical utility, and the communication among health-care providers.”<sup>4(p8)</sup>

Improvements in the analytic performance of HER2 testing in clinical practice since 2007 led the Panel to expand its focus beyond earlier concerns to reduce false-positive tests (and increase specificity).

## 2013 Update of the American Society of Clinical Oncology/College of American Pathologists Guideline for Human Epidermal Growth Factor Receptor 2 Testing: Impact on Immunohistochemistry-Negative Breast Cancers

### Reply to R. Bhargava et al and K. Lambein et al

On the basis of reanalysis of a small group of highly selected patients, Lambein et al,<sup>1</sup> in their correspondence to *Journal of Clinical Oncology*, assert that approximately 16% of breast cancers previously deemed to be human epidermal growth factor receptor 2

Table 1.

IHC 2007
Score
Score
Total
Abbrev

increase

## Histopathology

*Histopathology* 2014, 64, 609–615. DOI: 10.1111/his.12357

### REVIEW

## The updated ASCO/CAP guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice

Emad A Rakha, Jane Starczynski,<sup>1</sup> Andrew H S Lee & Ian O Ellis

<sup>1</sup>Division of Oncology, School of Medicine, University of Nottingham, Nottingham City Hospital, Nottingham, UK, and

<sup>2</sup>Department of Cellular Pathology, Birmingham Heartlands Hospital, Birmingham, UK

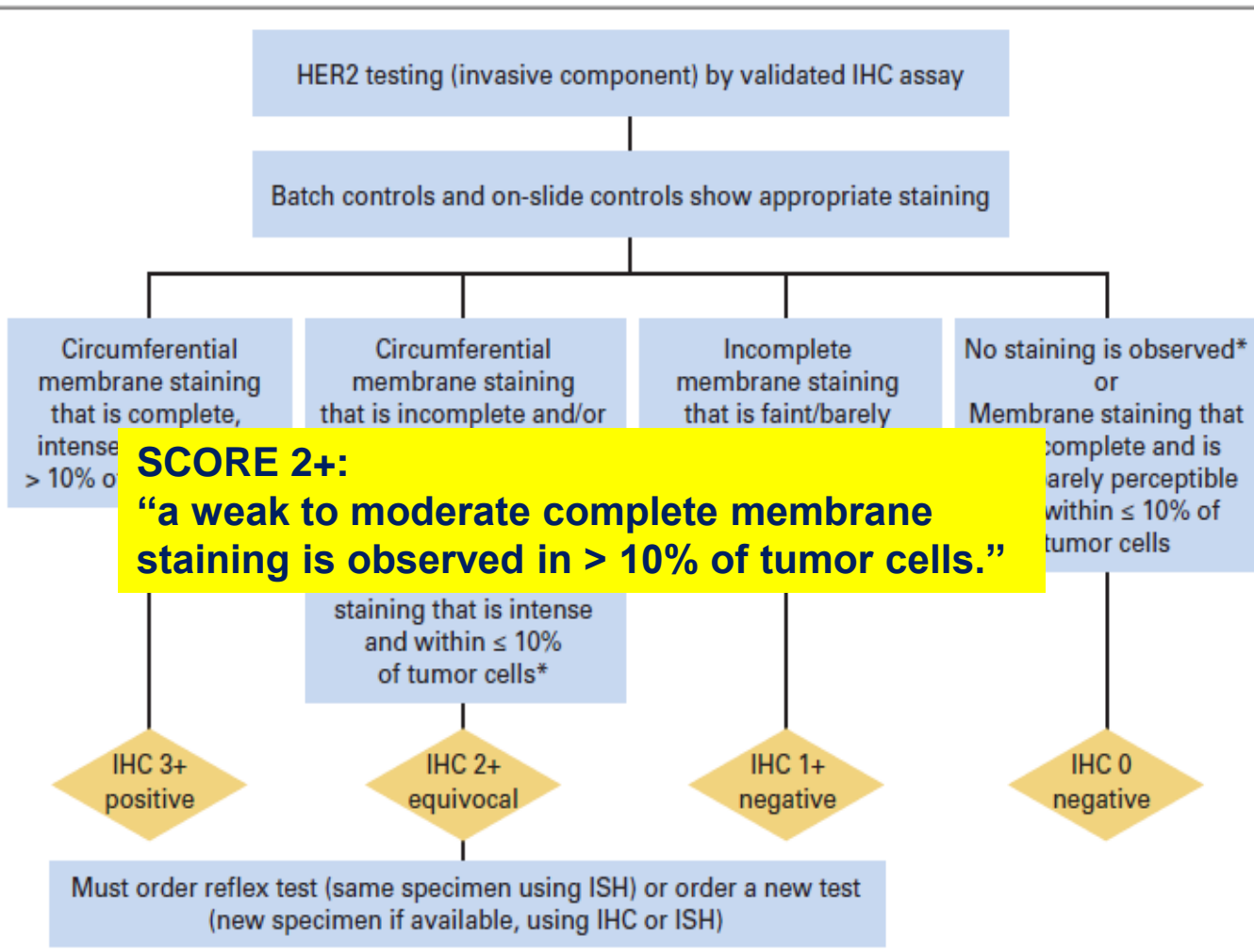
Reply to E.A. Rakha et al

Therefore, the Panel will be publishing two revisions in Figure 1. First, the definition for IHC 2+ in invasive breast cancer will now simply reflect the commonly accepted definition: “a weak to moderate complete membrane staining [that] is observed in > 10% of tumor cells.”<sup>5(p16)</sup> Second, discussions about possible uncommon IHC scenarios will be limited to the figure legend. The Figure 1 legend will be partly revised to read: “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 rare breast cancers (eg, micropapillary carcinomas) show IHC staining that is moderate to intense but incomplete (basolateral or lateral) and can be found to be HER2 amplified.<sup>6</sup> Another example describes circumferential membrane IHC staining that is intense but within  $\leq$  10% of tumor cells.”



# **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**



**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. Although categories of HER2 status by IHC can be created that are not covered by these definitions, in practice they are rare and if encountered should be considered IHC 2+ equivocal. ISH, in situ hybridization. NOTE: the final reported results assume that there is no apparent histopathologic discordance observed by the pathologist. (\*) Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.

colorazione incompleta (baso-laterale o laterale) moderata o intensa in >10% delle cellule (ex 1+)  
 colorazione completa intensa circonferenziale in <10% delle cellule (ex 0).

**Raccomandazioni GIPAM versione dicembre 2015**  
(elaborato nel 2013 aggiornato nel dicembre 2014 aggiornato nel dicembre 2015)

**Documento condiviso nell'ambito del GIPaM**  
(gruppo italiano dei patologi della mammella)

La reazione immunocitochimica con **anticorpo anti c-erbB2** oncoproteina (clone-kit-ditta) è risultata:

**1) POSITIVA (score 3+)**

Colorazione della membrana cellulare completa ed intensa, circonferenziale, in > 10% delle cellule di carcinoma invasivo (indicare la %)

**2) EQUIVOCA (score 2+)**

-colorazione della membrana cellulare completa, debole-moderata, in >10% delle cellule di carcinoma invasivo \*

\*Tenere presente che raramente si possono osservare le seguenti positività, per le quali è prudentiale dare uno score 2+ ed inviare in ISH:

-colorazione della membrana cellulare incompleta (baso-laterale o laterale), moderata/intensa in >10% delle cellule di carcinoma invasivo (RARO, ISTOTIPO MICROPAPILLARE)

-colorazione della membrana cellulare completa, intensa, nel 10% o meno delle cellule di carcinoma invasivo (RARO)

**CORRELAZIONE CON ALTRI PARAMETRI  
BIOLOGICI-MORFOLOGICI**



**Table 2.** Histopathologic Features Suggestive of Possible HER2 Test Discordance

## Criteria to Consider\*

New HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:

Histologic grade 1 carcinoma of the following types:

Infiltrating ductal or lobular carcinoma, ER and PgR positive

Tubular (at least 90% pure)

Mucinous (at least 90% pure)

Cribriform (at least 90% pure)

Adenoid cystic carcinoma (90% pure) and often triple negative

Similarly, 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 positive

Tubular (at least 90% pure)

Mucinous (at least 90% pure)

Cribriform (at least 90% pure)

Adenoid cystic carcinoma (90% pure) and often triple negative

If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed:

Tumor is grade 3

Amount of invasive tumor in the core biopsy is small

Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core

Core biopsy result is equivocal for HER2 after testing by both ISH and IHC

There is doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

# **Controlli di Qualità**

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*

**‘Non-classical’ *HER2* FISH results in breast cancer: a multi-institutional study** 

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

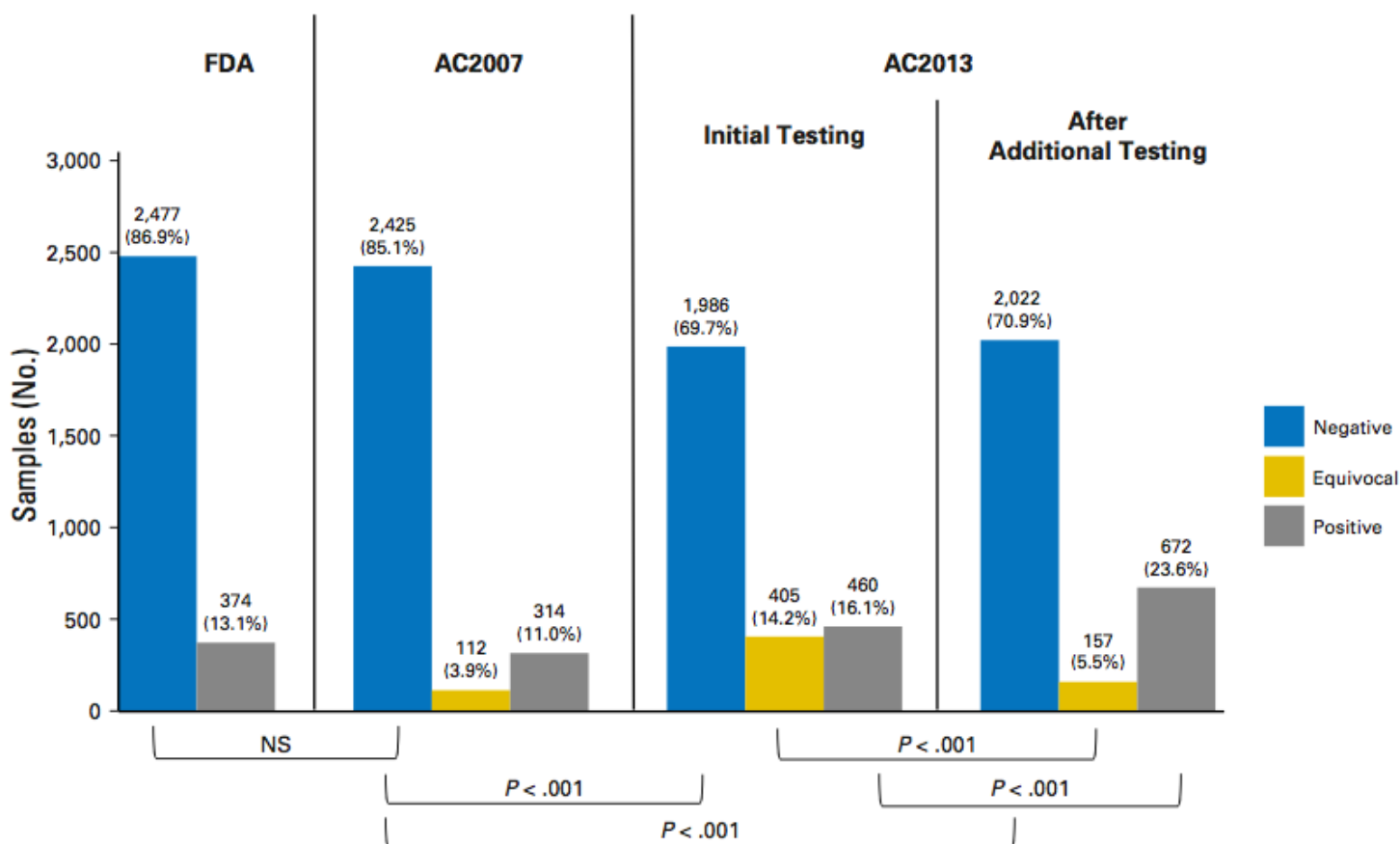
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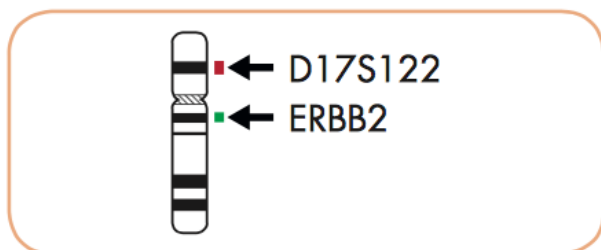
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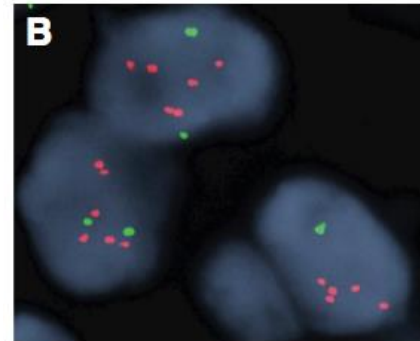
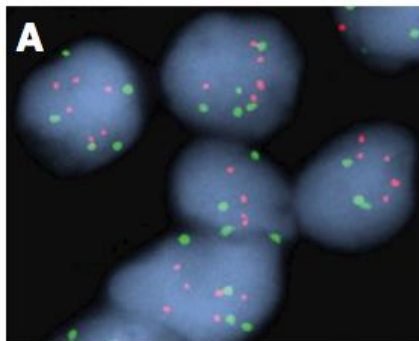
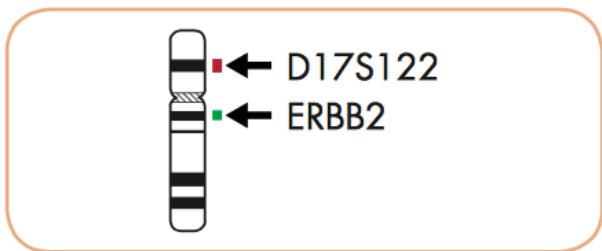
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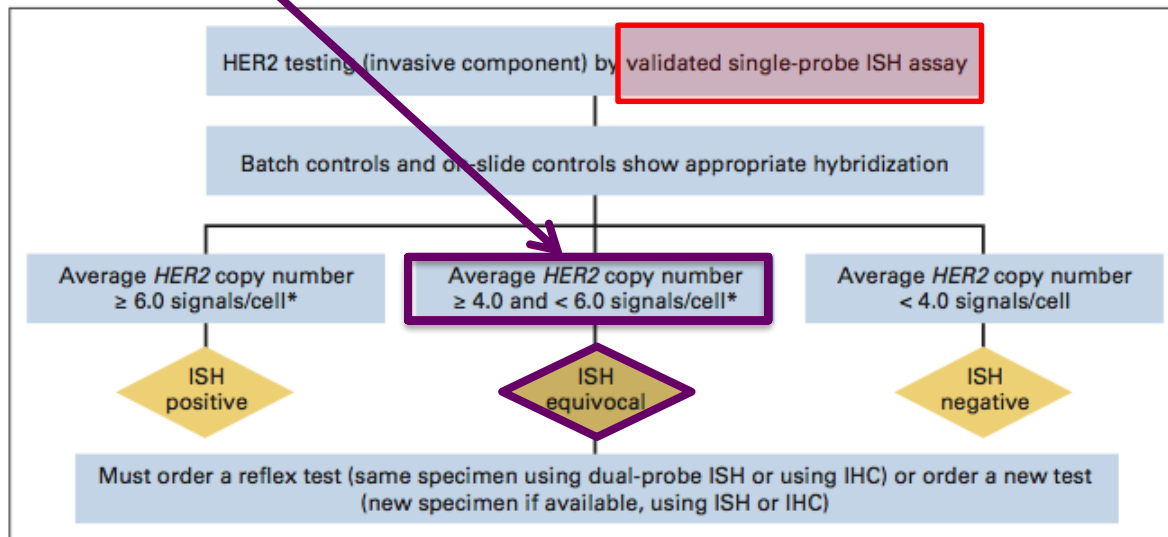
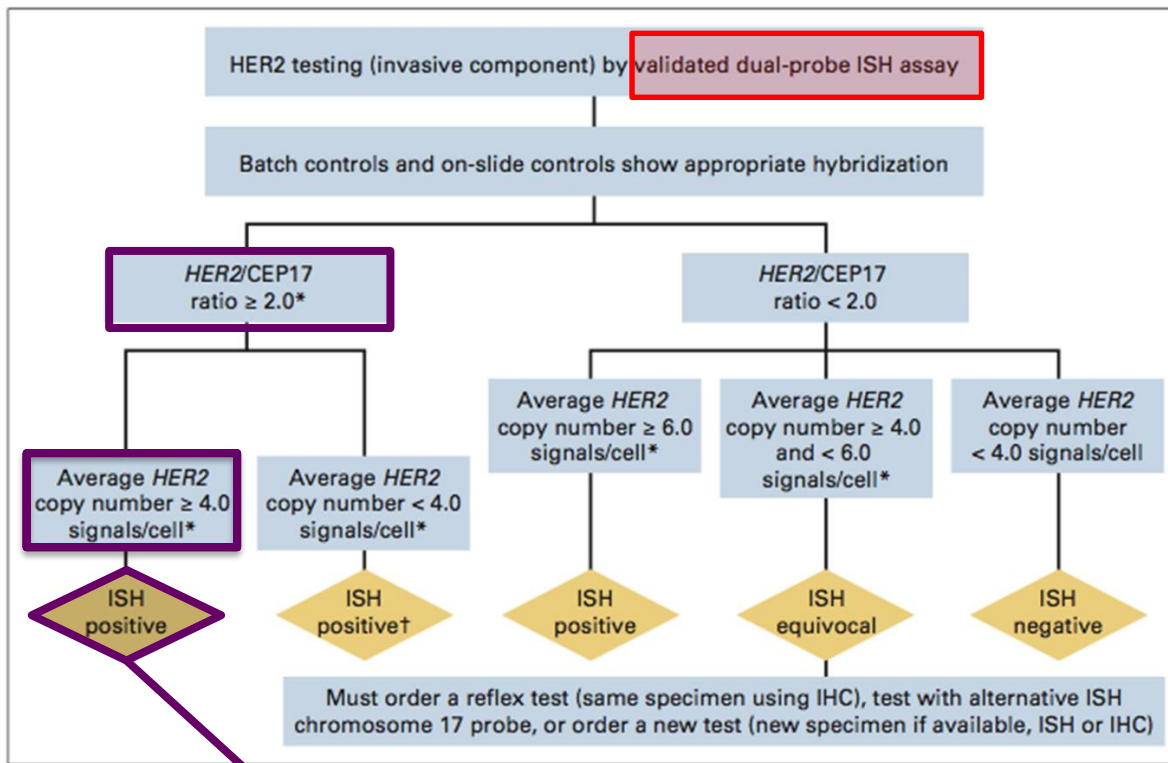
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Average *HER2* signal = 5.1  
 Average D17S122 signal = 5.8  
 $HER2:D17Z1 = 0.87$



Average *HER2* signal = 5.1  
 Average D17S122 signal = 1.7  
 $HER2:D17S122 = 3.0$



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

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







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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

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Category	Ratio	Mean HER2 signals/cell	Example
Classic Amplified	$\geq 2.0$	$\geq 6.0$	
Classic Non-amplified	$< 2.0$	$< 4.0$	
Equivocal	$< 2.0$	4.0 - 6.0	
Monosomy	$\geq 2.0$	$< 4.0$	
Co-amplified/Polysomy	$< 2.0$	$\geq 6.0$	
Low-amplified	$\geq 2.0$	4.0 - 6.0	



**Gruppo 1** = ISH positive classico



**Gruppo 5** = ISH negative – gruppo di controllo e paragone




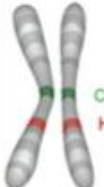




**Gruppo 4** = ISH Equivocal



**Gruppo 2** = ISH positive – low copy number/monosomy



**Gruppo 1** = ISH positive classico (Low Amplification)

Category	Ratio	Mean HER2 signals/cell	Example
Classic Amplified	$\geq 2.0$	$\geq 6.0$	
Classic Non-amplified	$< 2.0$	$< 4.0$	
Equivocal	$< 2.0$	4.0 - 6.0	
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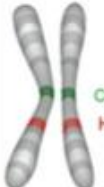




**Gruppo 4** = ISH Equivocal



**Gruppo 2** = ISH positive – low copy number/monosomy



**Gruppo 1** = ISH positive classico (Low Amplification)

Category	Ratio	Mean HER2 signals/cell	Example
Classic Amplified	$\geq 2.0$	$\geq 6.0$	 CEP17 HER2
Classic Non-amplified	$< 2.0$	$< 4.0$	 CEP17 HER2
Equivocal	$< 2.0$	4.0 - 6.0	 CEP17 HER2
Monosomy	$\geq 2.0$	$< 4.0$	 CEP17 HER2
Co-amplified/Polysomy	$< 2.0$	$\geq 6.0$	 CEP17 HER2
Low-amplified	$\geq 2.0$	4.0 - 6.0	 CEP17 HER2



**Gruppo 1 = ISH positive classico**



**Gruppo 5 = ISH negative – gruppo di controllo e paragone**



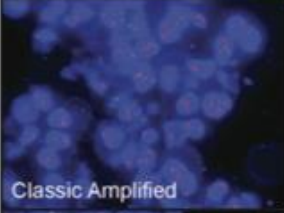

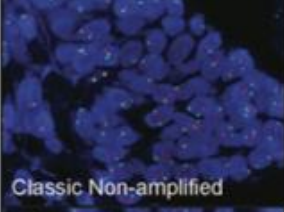

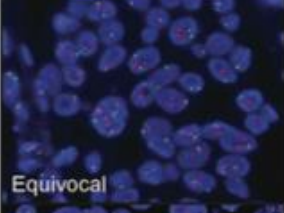

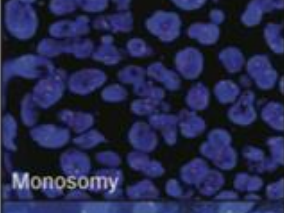

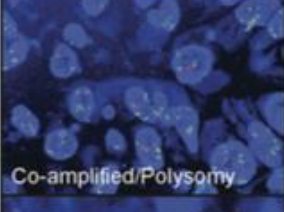

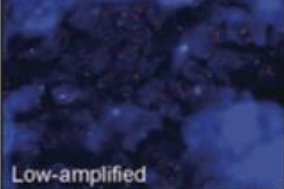

**Gruppo 4 = ISH Equivocal**

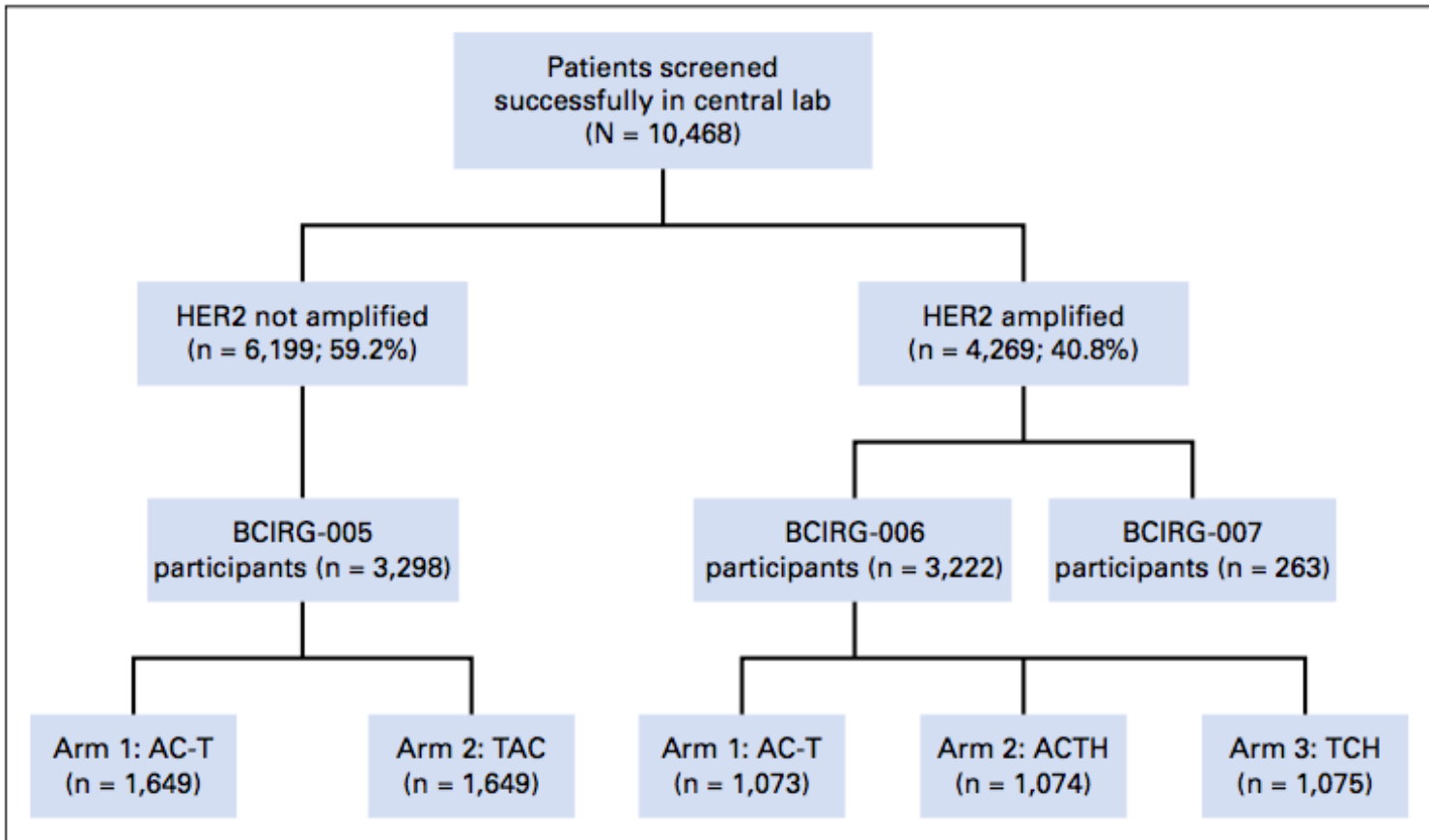








**Gruppo 2 = ISH positive – low copy number/monosomy**



**Gruppo 1 = ISH positive classico (Low Amplification)**







Category	Ratio	Mean HER2 signals/cell	Example
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 <p>Equivocal</p>	$< 2.0$	4.0 - 6.0	 <p>CEP17 HER2</p>
 <p>Monosomy</p>	$\geq 2.0$	$< 4.0$	 <p>CEP17 HER2</p>
 <p>Co-amplified/Polysomy</p>	$< 2.0$	$\geq 6.0$	 <p>CEP17 HER2</p>
 <p>Low-amplified</p>	$\geq 2.0$	4.0 - 6.0	 <p>CEP17 HER2</p>



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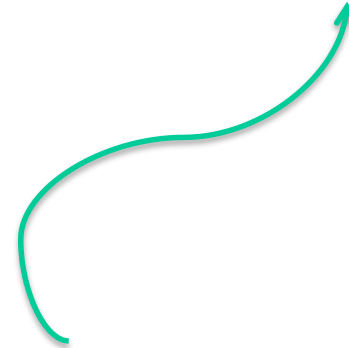


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Low-amplified	$\geq 2.0$	4.0 - 6.0	 CEP17 HER2



**Gruppo 5** = ISH negative – control group for comparison









**Supplemental Table S1. 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 <i>HER2</i> copies >6.0)	<i>HER2</i> BCIRG FISH Status	Mean of average <i>HER2</i> copy numbers	<b>HER2 Protein by 10H8-IHC score</b>				
			IHC 0	IHC 1+	IHC 2+	IHC 3+	
Group 3A	<b>Amplified</b>	Average 16.38	1 (17%)	0 (0%)	3 (50%)	2 (33%)	6 (24%)
Group 3N	<b>Not Amplified</b>	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 being IHC 2+/3+ compared with 89% of Group 3N being 0/1+ (p=0.002, Fisher's exact test).



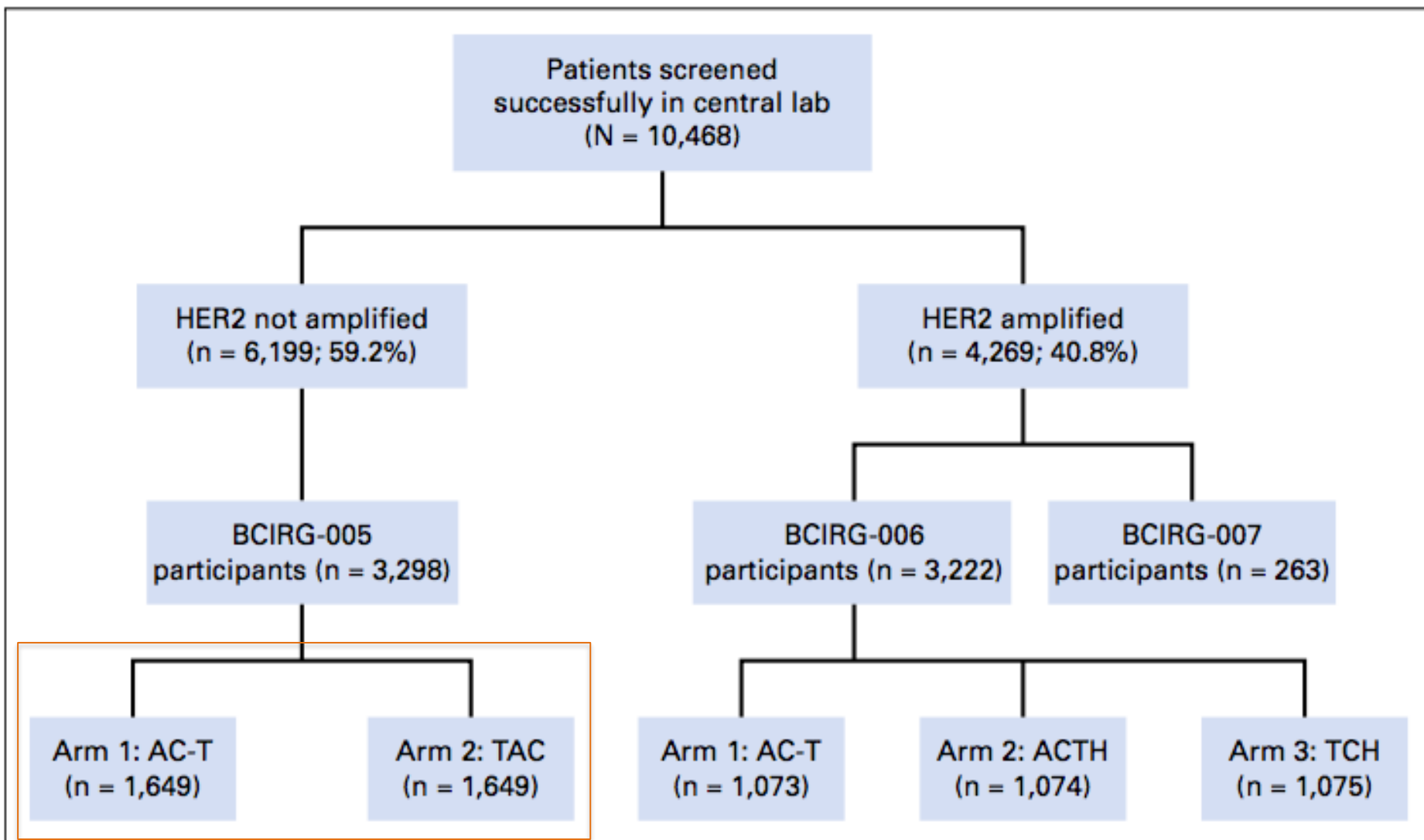
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Low-amplified	$\geq 2.0$	4.0 - 6.0	 CEP17 HER2



**Gruppo 5** = ISH negative – control group for comparison

**Gruppo 4** = ISH Equivocal





# Conclusioni

Area grigia di interpretazione da parte del patologo e del biologo molecolare

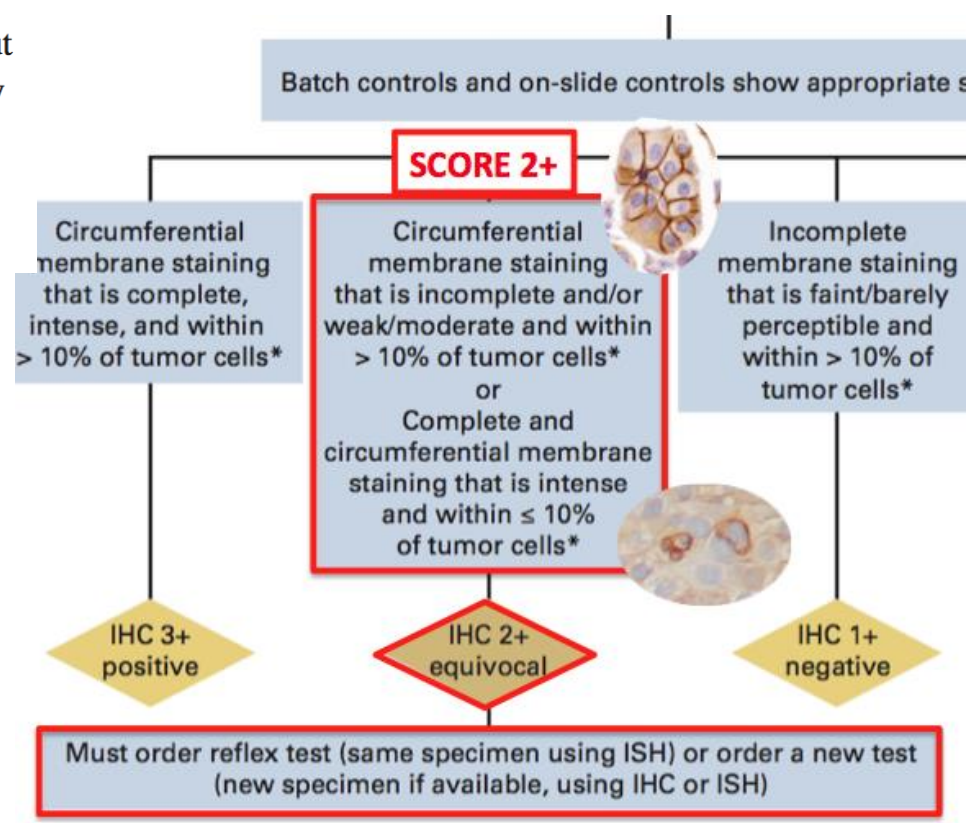
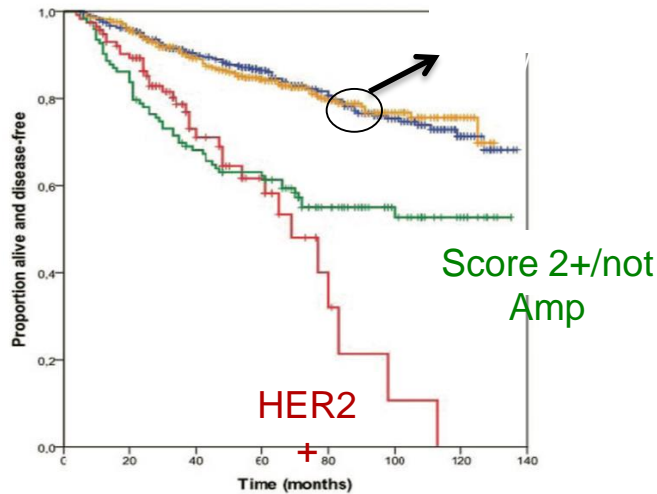
➤ Score 2+

Casi che vanno in discussione con Oncologo

- Dati ISH e dati IHC
- Dati clinicopatologici

# Moderate Immunohistochemical Expression of HER-2 (2+) Without *HER-2* Gene Amplification Is a Negative Prognostic Factor in Early Breast Cancer

VALENTINA ROSSI,<sup>a</sup> IVANA SAROTTO,<sup>b</sup> FURIO MAGGIOROTTO,<sup>c</sup> PAOLA BERCHIALLA,<sup>d</sup>  
 FRANZISKA KUBATZKI,<sup>c</sup> NICOLETTA TOMASI,<sup>c</sup> STEFANIA REDANA,<sup>a</sup> ROSSELLA MARTINELLO,<sup>a</sup>  
 GIORGIO VALABREGA,<sup>a</sup> MASSIMO AGLIETTA,<sup>a</sup> RICCARDO PONZONE,<sup>c</sup> FILIPPO MONTEMURRO<sup>a,f</sup>



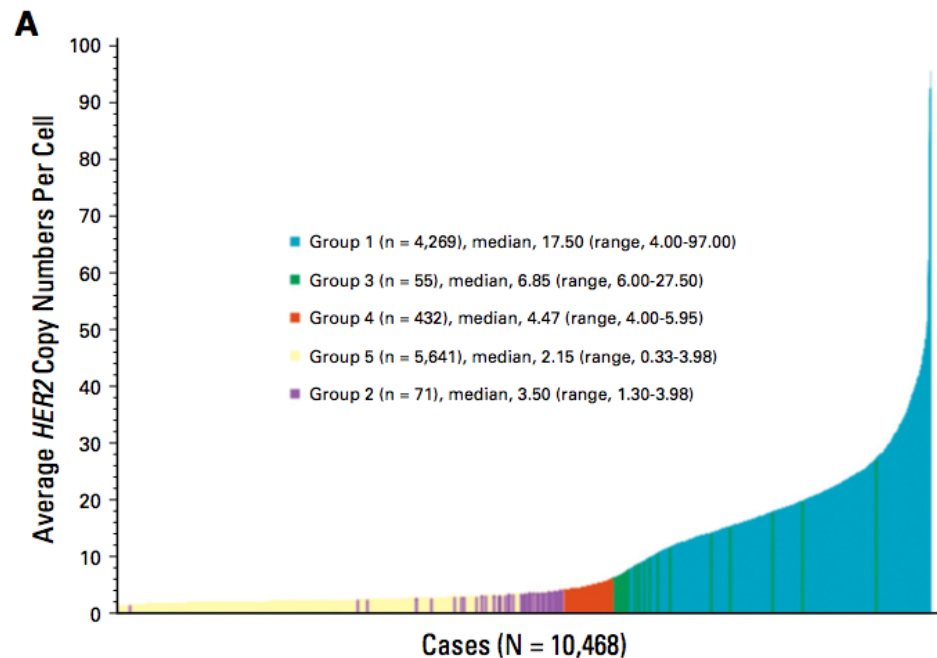
## HER2 Equivocal Status in Early Breast Cancer Is Not Associated with Higher Risk of Recurrence

CARMEN CRISCITIELLO<sup>1</sup>, VINCENZO BAGNARDI<sup>2,3</sup>, GIUSEPPE VIALE<sup>4</sup>, DAVIDE DISALVATORE<sup>2</sup>,  
 NICOLE ROTMENSZ<sup>1</sup>, ANGELA ESPOSITO<sup>1</sup>, ARON GOLDBIRSCHE<sup>5</sup> and GIUSEPPE CURIGLIANO<sup>1</sup>

We found no significant association between risk of recurrence and HER2 equivocal testing in patients with early breast cancer. In subgroup analysis, a significant interaction between HER2/CEP17 ratio and nodal involvement was observed ( $p=0.02$ ). Patients with HER2 equivocal status have no significant risk of recurrence. In our analysis IHC HER2 2+ status with HER2/CEP17 ratio <2.0 has no impact on prognosis of patients with early breast cancer.

# 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, H el ene 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



# ISH groups and IHC

PathVysion <i>HER2</i> FISH Assay		HercepTest IHC Score*					Total	P†	ASCO-CAP FISH Group
<i>HER2</i> FISH Ratio	Average <i>HER2</i> Copy Number per Cell	0	1+	2+	3+				
Comparison of <i>HER2</i> FISH Ratios and Copy Numbers With <i>HER2</i> Protein by HercepTest Scores According to ASCO-CAP Groupings									
< 2.0	< 4.0	1,988 (94.1%)	114 (5.4%)	10 (0.5%)	1 (0.05%)	2,113 (100%)	< .0001§	Group 5	
	≥ 4.0-5.99	105 (78.4%)	21 (15.7%)	7 (5.2%)	1 (0.7%)	134 (100%)	< .0001§	Group 4	
	≥ 6.0	5 (55.6%)	2 (22.2%)	1 (11.1%)	1 (11.1%)	9 (100%)	.3881§	Group 3	
	Total	2,098 (93.0%)	137 (6.1%)	18 (0.8%)	3 (0.1%)	2,256 (100%)	< .0001§	Groups 3-5	
≥ 2.0	< 4.0	24 (68.6%)	8 (22.9%)	3 (8.6%)	0 (0%)	35 (100%)	< .0007	Group 2	
	≥ 4.0-5.99	65 (65.7%)	22 (22.2%)	10 (10.1%)	2 (2.0%)	99 (100%)	< .0001	Group 1	
	≥ 6.0	175 (9.0%)	242 (12.5%)	561 (28.9%)	963 (49.6%)	1,941 (100%)	< .0001¶	Group 1	
	Total	264 (12.7%)	272 (13.1%)	574 (27.7%)	965 (46.5%)	2,075 (100%)	< .0001#		
Total		2,362	409	592	968	4,331			

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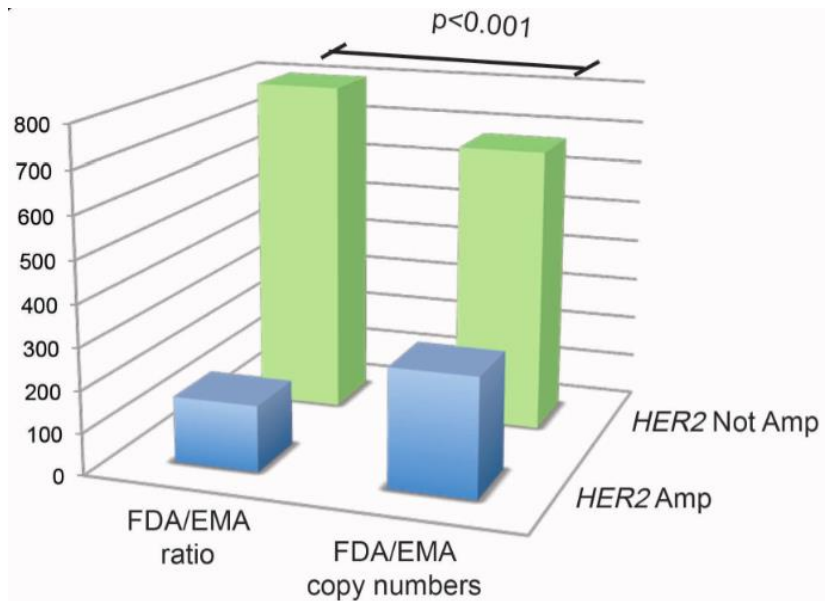
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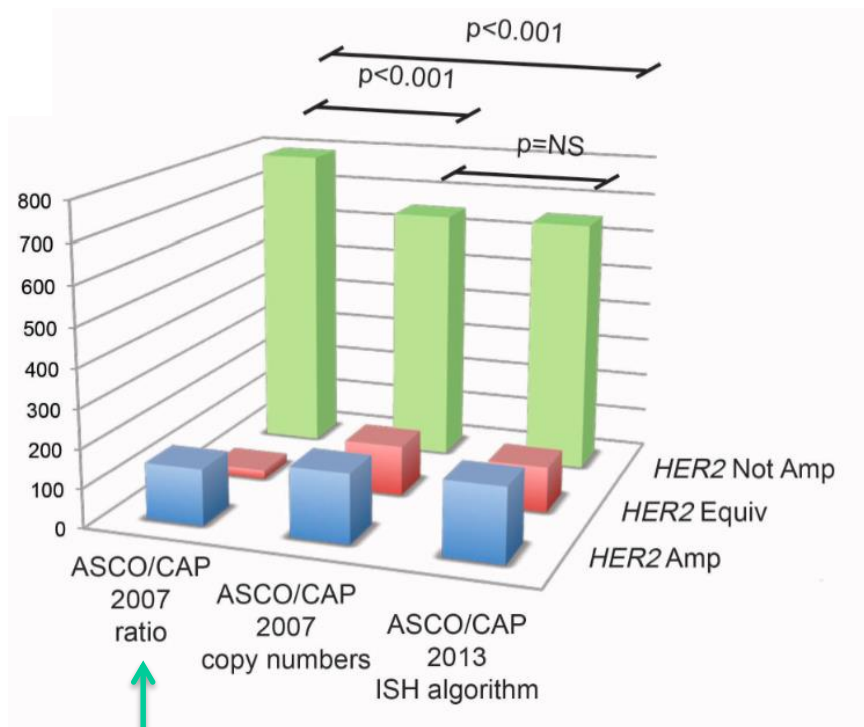


### Gene Status in *HER2* Equivocal Breast Carcinomas: Impact of Distinct Recommendations and Contribution of a Polymerase Chain Reaction-Based Method

ANNA SAPINO,<sup>a,b</sup> FRANCESCA MALETTA,<sup>a,b</sup> LUDOVICA VERDUN DI CANTOGNO,<sup>b</sup> LUGIA MACRÌ,<sup>b</sup> CRISTINA BOTTA,<sup>a</sup> PATRIZIA GUGLIOTTA,<sup>a</sup> MARIA STELLA SCALZO,<sup>b</sup> LAURA ANNARATONE,<sup>a</sup> DAVIDE BALMATIVOLA,<sup>a,b</sup> FRANCESCA PIETRIBIASI,<sup>c</sup> PAOLO BERNARDI,<sup>d</sup> RICCARDO ARISIO,<sup>b</sup> LAURA VIBERTI,<sup>e</sup> STEFANO GUZZETTI,<sup>f</sup> RENZO ORLASSINO,<sup>g</sup> CRISTIANA ERCOLANI,<sup>h</sup> MARCELLA MOTTOLESE,<sup>h</sup> GIUSEPPE VIALE,<sup>h,j</sup> CATERINA MARCHIÒ<sup>a,b</sup>



Highest AMP  
29.5%



Lowest AMP  
15%