



# **BIOMARCATORI: Quali Biomarcatori, la refertazione**

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# FATTORI PROGNOSTICI

## Fattori morfologici:

- Stato linfonodale,
  - Diametro del tumore,
  - Grado
  - Tipo Istologico
  - Indice proliferativo (mitosi)
  - Invasione vascolare
- } **STADIO**

## Fattori immunofenotipici:

Recettori Ormonale (ER, PR)

HER2

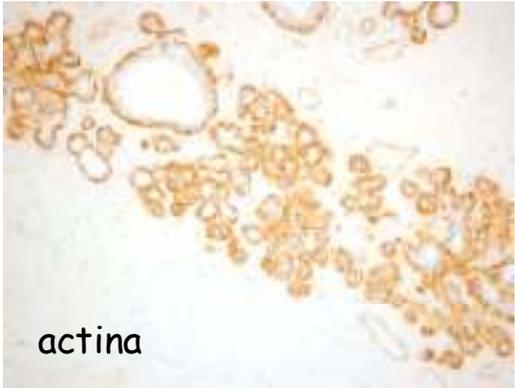
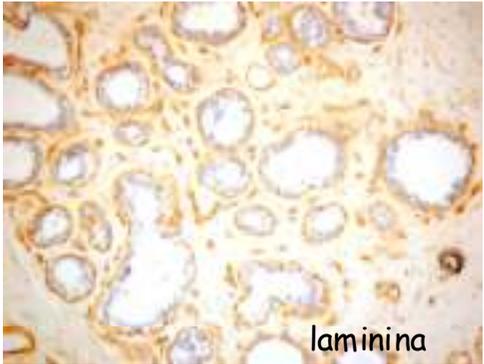
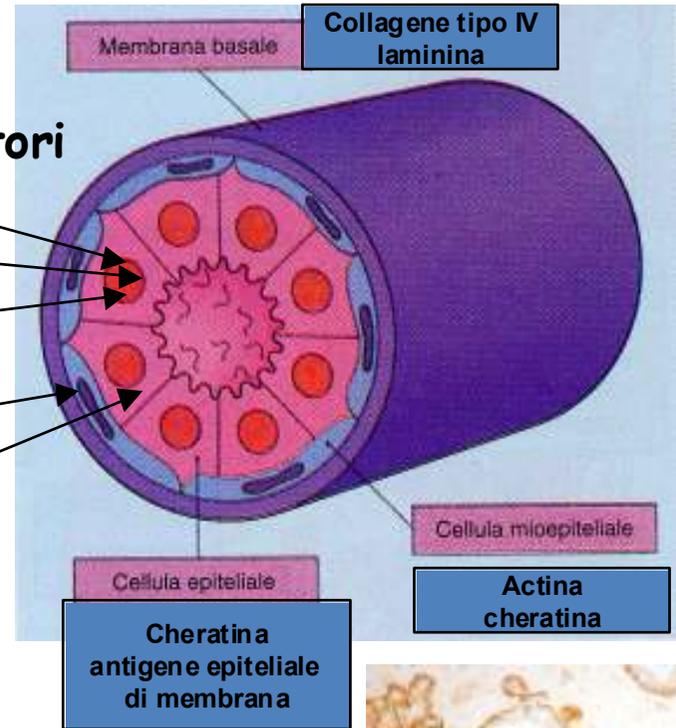
ki67

# Struttura microscopica

ormoni

Proliferazione  
differenziazione

- estrogeno
  - progesterone
  - prolattina
  - ossitocina
  - androgeni
- recettori



HER2: Recettori overespressi in caso di patologia neoplastica

# FATTORI PREDITTIVI

**Fattori immunofenotipici**

**Recettori Ormonale (ER, PR)**

**HER2**

**KI67**

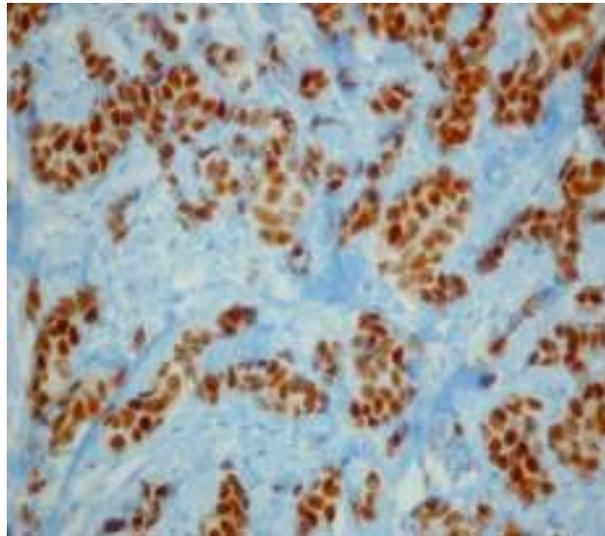
**Ormonoterapia**

**Terapia con Trastuzumab**

**Chemioterapia**

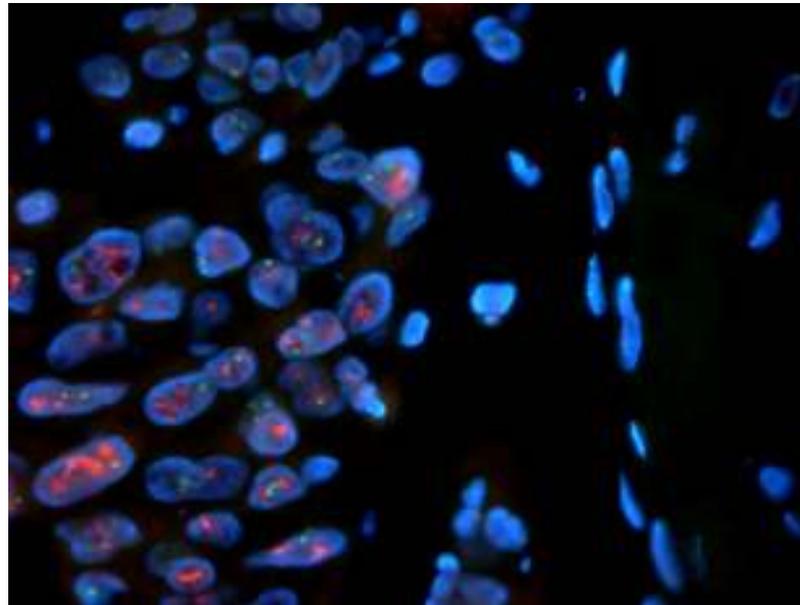
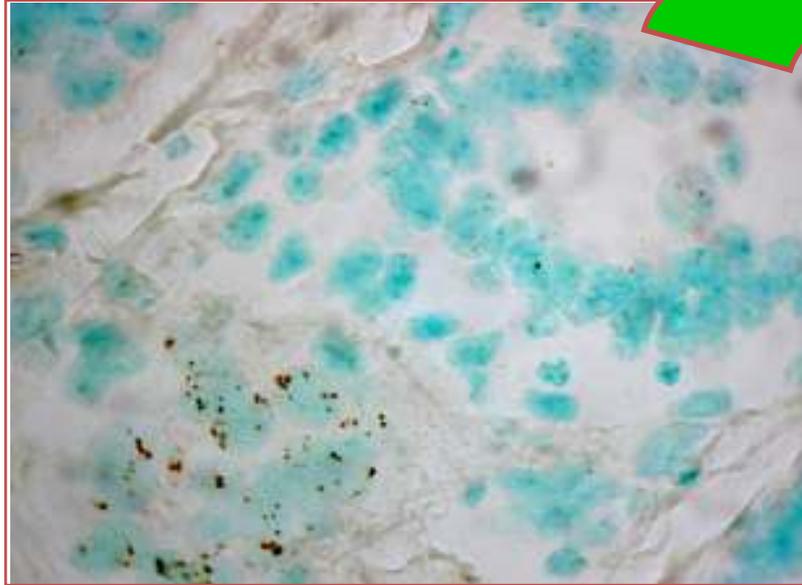
American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer

The Panel recommends a cutoff of a minimum of **1%** of tumor cells positive for ER/PgR for a specimen to be considered positive..

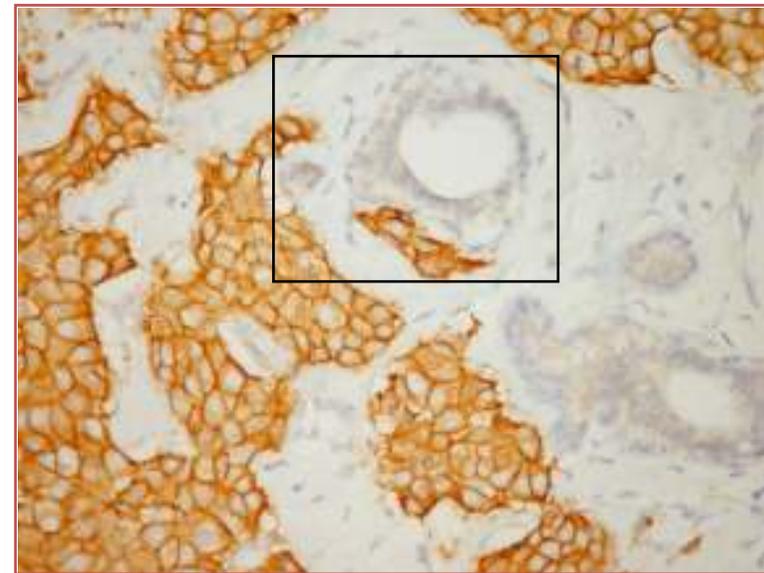


*Journal of Clinical Oncology* and the *Archives of Pathology & Laboratory Medicine*. Copyright © 2010

*The gene*



*The antigen*



**Protein over-expression**

Intrinsic Subtype (1)	Clinico-pathologic definition	Type of therapy
Luminal A	<p>'Luminal A'</p> <p>ER and/or PgR positive (76)</p> <p>HER2 negative (77)</p> <p>Ki-67 low (&lt;14%)</p>	<p>SOLO TERAPIA ENDOCRINA</p>
Luminal B <sup>*</sup>	<p>'Luminal B (HER2 negative)'</p> <p>ER and/or PgR positive</p> <p>HER2 negative</p> <p>Ki-67 high</p>	<p>TERAPIA ENDOCRINA + CHEMIOTERAPIA</p>
	<p>'Luminal B (HER2 positive)'</p> <p>ER and/or PgR positive</p> <p>Any Ki-67</p> <p>HER2 over-expressed or amplified</p>	<p>CHEMIOTERAPIA + TERAPIA ANTI-HER2 + TERAPIA ENDOCRINA</p>
Erb-B2 overexpression	<p>'HER2 positive (non luminal)'</p> <p>HER2 over-expressed or amplified</p> <p>ER and PgR absent</p>	<p>CHEMIOTERAPIA + TERAPIA ANTI-HER2</p>
'Basal-like'	<p>'Triple negative (ductal)'</p> <p>ER and PgR absent</p> <p>HER2 negative</p>	<p>SOLO CHEMIOTERAPIA</p>

# Quando vengono eseguiti

- **Tutti** i carcinomi di nuova diagnosi
  - i) su pezzo operatorio
  - ii) su agobiopsia per terapia neoadiuvante
- Recidive e Metastasi
- Carcinoma in situ: solo ER e PR



# ER e PgR - Linee Guida ASCO/CAP

## ➤ Test clinicamente validati:

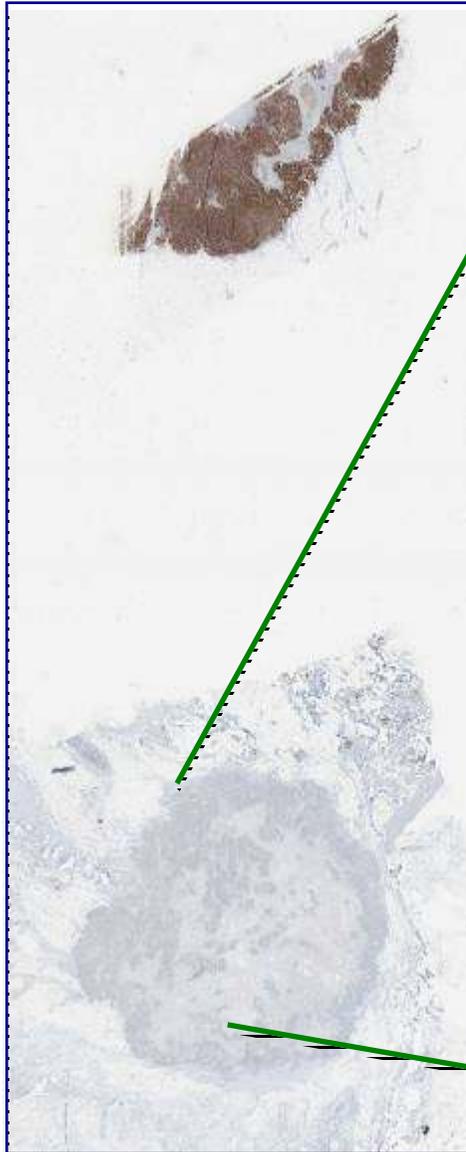
- IHC (diversi anticorpi)

La selezione degli anticorpi per le reazioni immunoistochimiche per ER e PgR dovrebbe essere **limitata a quei reagenti che abbiano una SPECIFICITA' e una SENSIBILITA' ben note e che siano stati VALIDATI CLINICAMENTE**, dimostrando una buona correlazione con la prognosi delle pazienti in letteratura

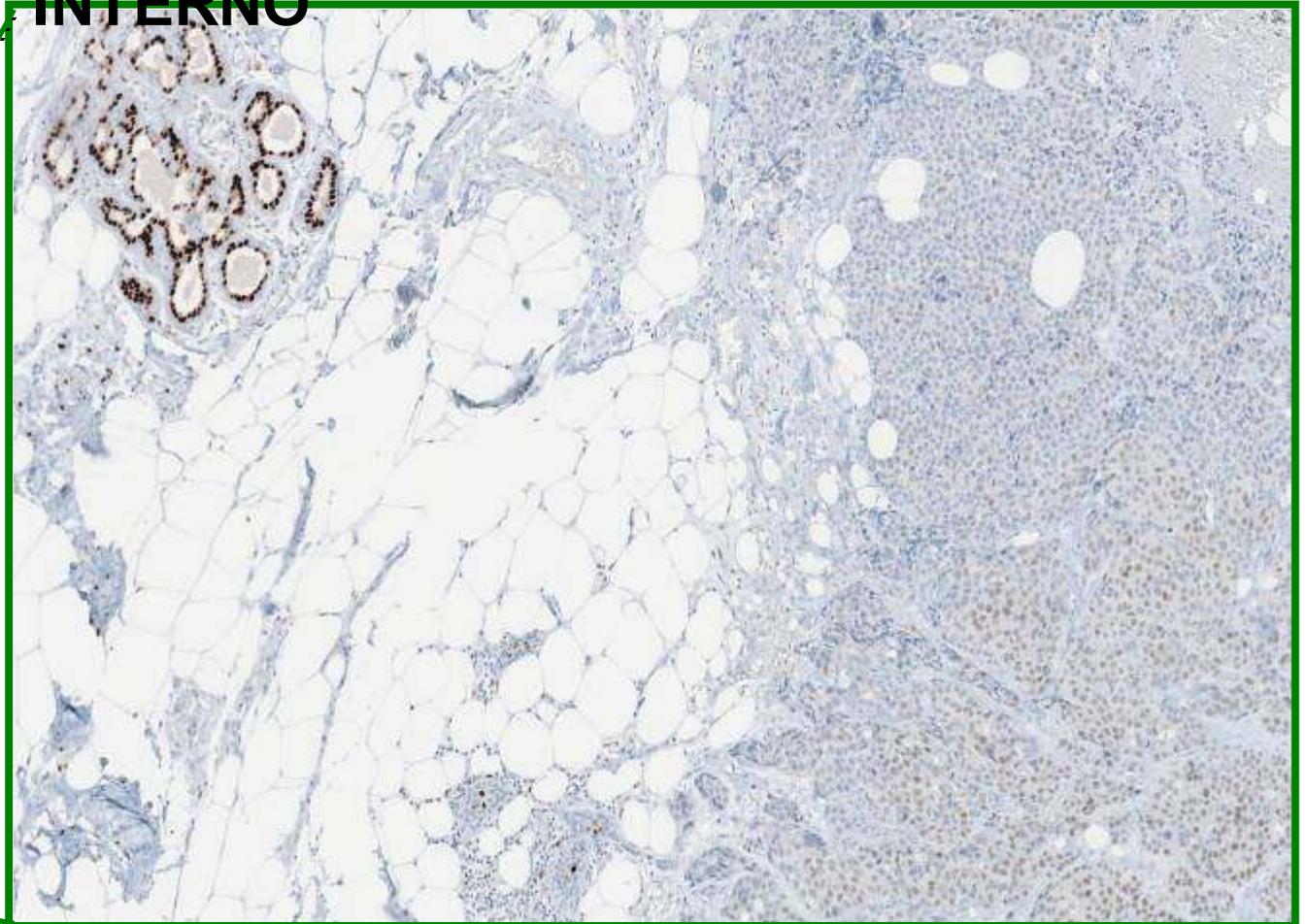
**Table 3. Well-Validated Assays for Evaluating Estrogen Receptor and Progesterone Receptor in Breast Cancer by Immunohistochemistry**

Reference	Primary Antibody	Cut Point for "Positive"
Estrogen receptor		
Harvey et al, 1999 <sup>8</sup>	6F11	Allred score $\geq 3$ (1% to 10% weakly positive cells)
Regan et al, 2006 <sup>9</sup> , Viale et al, 2007 <sup>13</sup> , Viale et al, 2008 <sup>14</sup>	1D5	1% to 9% (low) and $\geq 10%$ (high)
Cheang et al, 2006 <sup>15</sup>	SP1	$\geq 1%$
Phillips et al, 2007 <sup>16</sup>	ER.2.122 + 1D5 (cocktail)	Allred score $\geq 3$ (1% to 10% weakly positive cells)
Dowsett et al, 2008 <sup>17</sup>	6F11	H score $> 1$ ( $\geq 1%$ )
Progesterone receptor		
Mohsin et al, 2004 <sup>10</sup>	1294	Allred score $\geq 3$ (1% to 10% weakly positive cells)
Regan et al, 2006 <sup>9</sup> , Viale et al, 2007 <sup>13</sup> , Viale et al, 2008 <sup>14</sup>	1A6	1% to 9% (low) and $\geq 10%$ (high)
Phillips et al, 2007 <sup>16</sup>	1294	Allred score $\geq 3$ (1% to 10% weakly positive cells)
Dowsett et al, 2008 <sup>17</sup>	312	$\geq 10%$

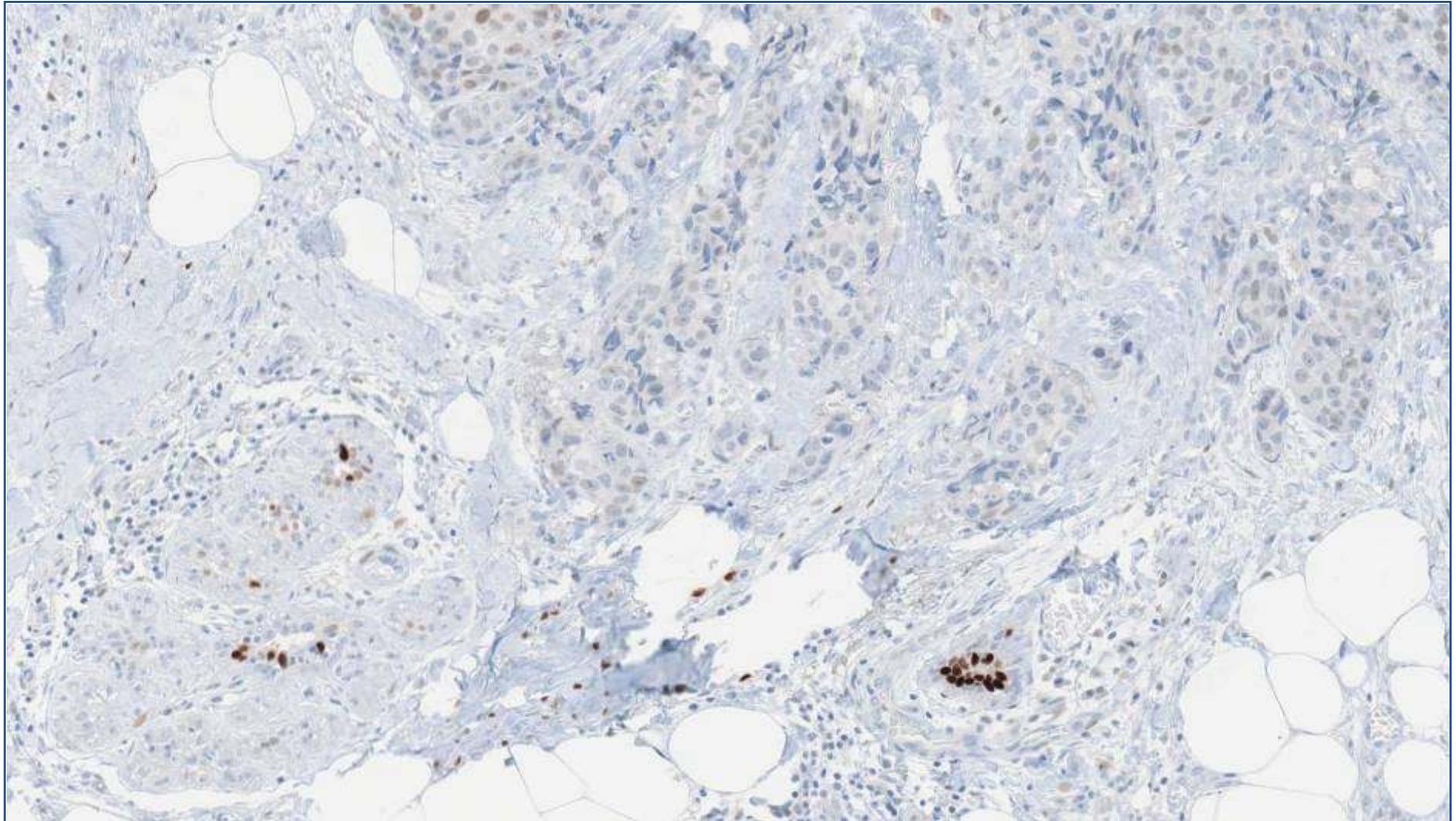
# ER e PgR - Linee Guida ASCO/CAP



**CONTROLLO ESTERNO E CONTROLLO INTERNO**



# ER e PgR - Linee Guida ASCO/CAP



# ER e PgR - Linee Guida ASCO/CAP

## ➤ Refertazione

1 informazione **necessaria**

**Percentuale** cellule colorate

Table 1. Summary of Guideline Recommendations for ER and PgR Testing by IHC in Breast Cancer Patients

	Recommendation
ER and PgR testing	Positive for ER or PgR if finding of $\geq 1\%$ of tumor cell nuclei are immunoreactive. Negative for ER or PgR if finding of $< 1\%$ of tumor cell nuclei are immunoreactive in the presence of evidence that the sample can express ER or PgR (positive intrinsic controls are seen). Uninterpretable for ER or PgR if finding that no tumor nuclei are immunoreactive and that internal control elements present in the sample or separately submitted from the same sample show any nuclear staining.

# ER e PgR - Linee Guida ASCO/CAP

## ➤ Refertazione

1 informazione **necessaria**

**Percentuale** cellule colorate

Alcune informazioni **opzionali**

**Intensità**

lieve  
moderata  
intensa

**Score composito**

Tipo Allred score o H score  
...

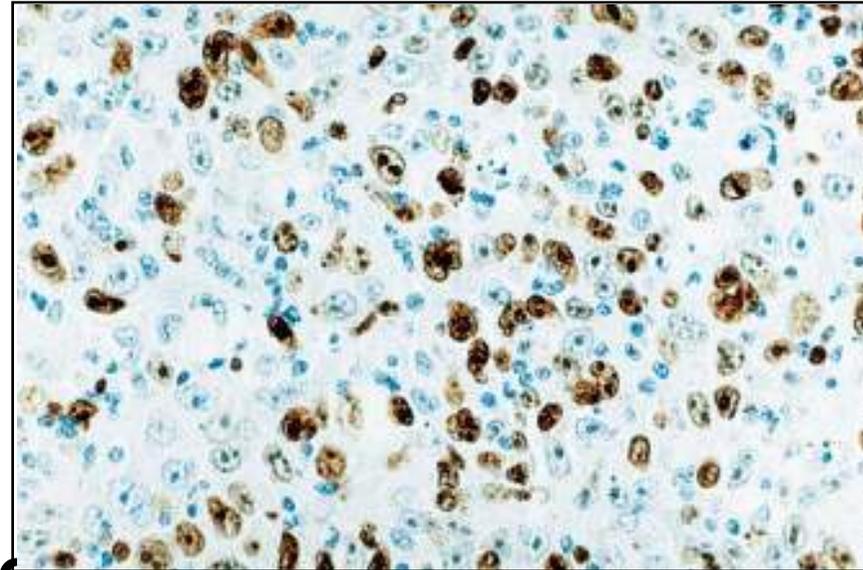
## ***Interpretazione finale:***

- recettori positivi
- recettori negativi
- recettori non interpretabili

# Indice proliferativo – Ki67

## Come si determina

Immunoistochimica (clone MIB1)

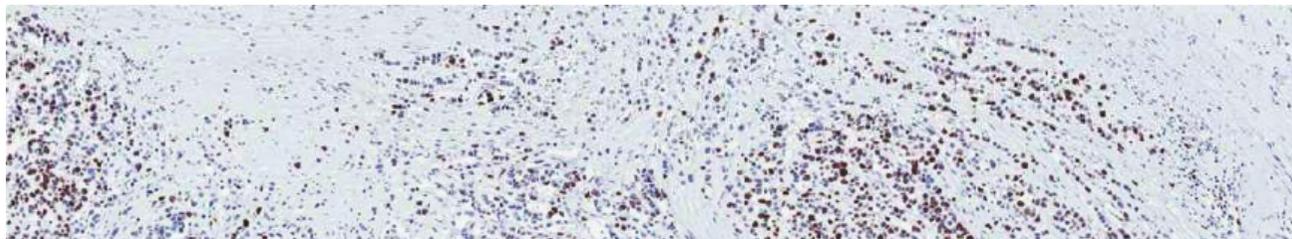


## Come si legge



Valore di percentuale di cellule positive

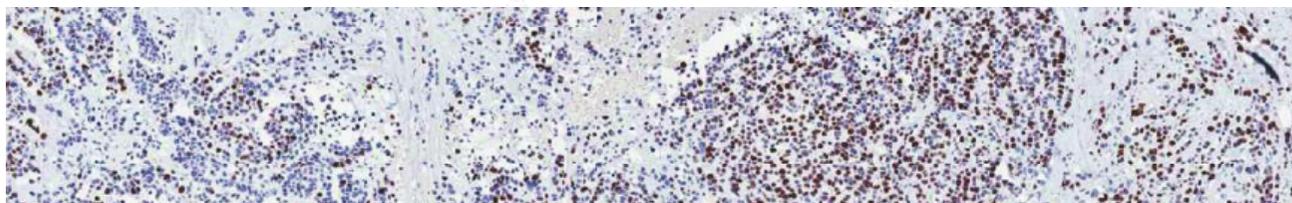
# Indice proliferativo – Ki67



## Box 1. Recommendations for Ki67 assessment in breast cancer

### Interpretation and scoring

- In full sections, at least three high-power (x40 objective) fields should be selected to represent the spectrum of staining seen on initial overview of the whole section.
- For the purpose of prognostic evaluation, the invasive edge of the tumor should be scored.
- If pharmacodynamic comparisons must be between core cuts and sections from the excision, assessment of the latter should be across the whole tumor.
- If there are clear hot spots, data from these should be included in the overall score.
- Only nuclear staining is considered positive. Staining intensity is not relevant.
- Scoring should involve the counting of at least 500 malignant invasive cells (and preferably at least 1000 cells) unless a protocol clearly states reasons for fewer being acceptable.
- Image analysis methods for Ki67 remain to be proven for use in clinical practice.



# Indice proliferativo – Ki67

J Natl Cancer Inst;2013;105:1897–1906

## **An International Ki67 Reproducibility Study**

Mei-Yin C. Polley, Samuel C. Y. Leung, Lisa M. McShane, Dongxia Gao, Judith C. Hugh, Mauro G. Mastropasqua, Giuseppe Viale, Lila A. Zabaglo, Frédérique Penault-Llorca, John M.S. Bartlett, Allen M. Gown, W. Fraser Symmans, Tammy Piper, Erika Mehl, Rebecca A. Enos, Daniel F. Hayes, Mitch Dowsett, Torsten O. Nielsen, on behalf of the International Ki67 in Breast Cancer Working Group of the Breast International Group and North American Breast Cancer Group

Manuscript received April 2, 2013; revised September 3, 2013; accepted September 16, 2013.

**Correspondence to:** Torsten Nielsen, MD, PhD, FRCPC, University of British Columbia Pathology and Laboratory Medicine, Anatomical Pathology, JP 1401, Vancouver Hospital & Health Sciences Centre, 855 W 12th Ave, Vancouver, BC V5Z 1M9, Canada (e-mail: [torsten@mail.ubc.ca](mailto:torsten@mail.ubc.ca)).

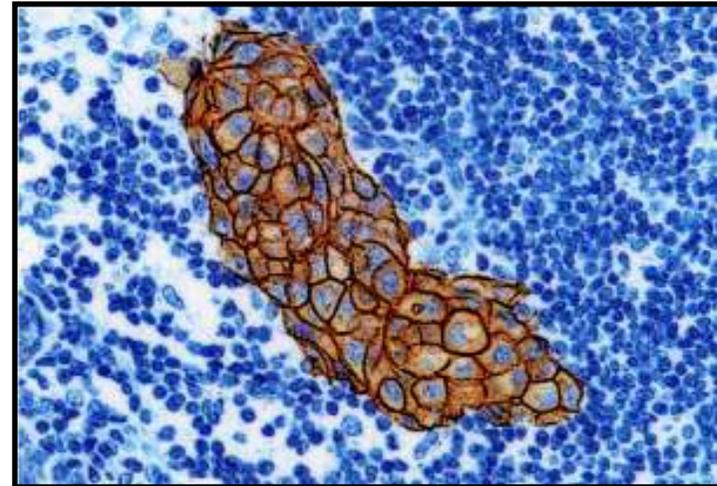
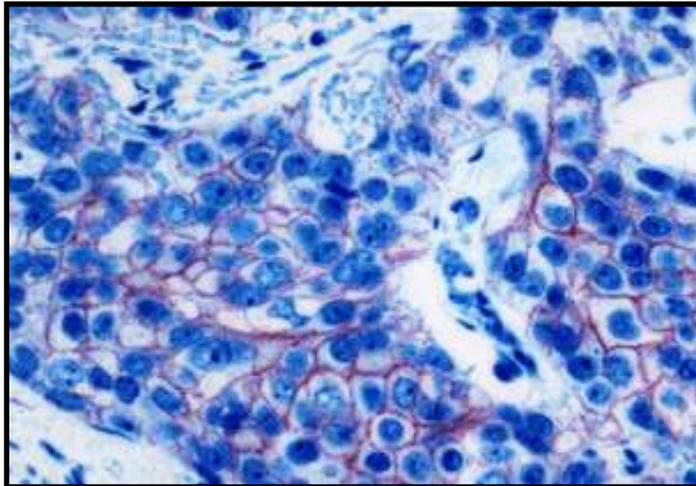
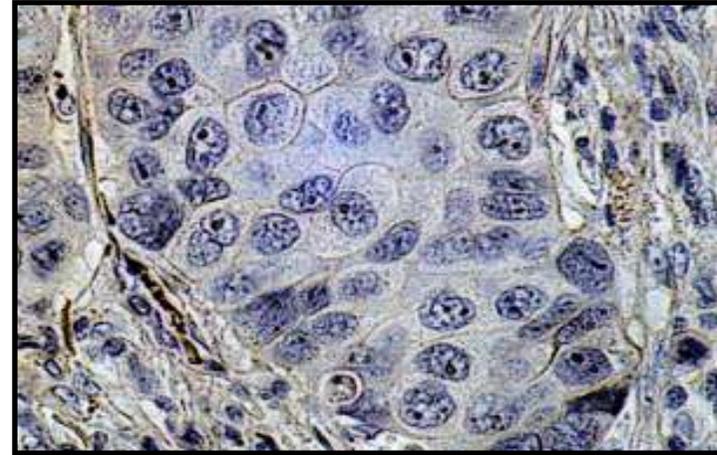
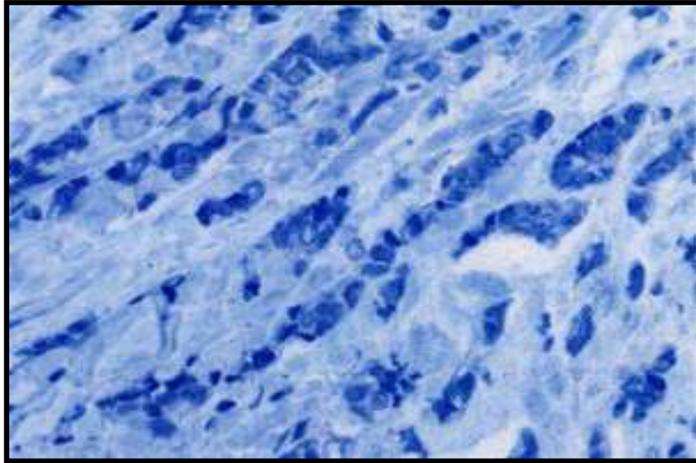
Eight laboratories received 100 breast cancer cases arranged into 1-mm core tissue microarrays

One set stained by the participating laboratory and one set stained by the central laboratory, both using antibody MIB-1.

Intralaboratory reproducibility was high (ICC = 0.94; 95% CI = 0.93 to 0.97).

**Interlaboratory reproducibility was only moderate** (central staining: ICC = 0.71, 95% CI = 0.47 to 0.78; local staining: ICC = 0.59, 95% CI = 0.37 to 0.68).

# HER2: come si presenta



# HER2: test approvati da FDA

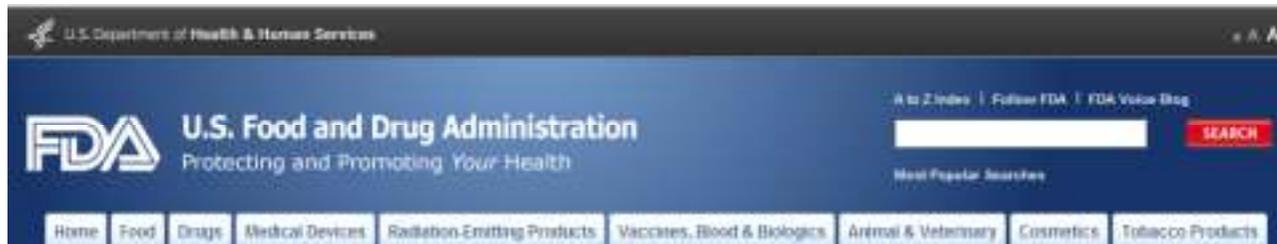


## 5.5 HER2 Testing

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for Herceptin therapy because these are the only patients studied and for whom benefit has been shown. Due to differences in tumor histopathology, use FDA-approved tests for the specific tumor type (breast or gastric/gastroesophageal adenocarcinoma) to assess HER2 protein overexpression and HER2 gene amplification. Tests should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of suboptimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

Several FDA-approved commercial assays are available to aid in the selection of breast cancer and metastatic gastric cancer patients for Herceptin therapy. Users should refer to the package inserts of specific assay kits for information on the Intended Use, and the validation and performance of each

# HER2: test approvati da FDA



**HercepTest** (Dako Denmark A / S, Glostrup, Denmark),

**PATHWAY HER2** (clone **4B5**; Ventana Medical Systems Inc., Tucson, AZ, USA),

**Oracle HER2** (clone **CB11**; Leica Microsystems GmbH, Wetzlar, Germany).

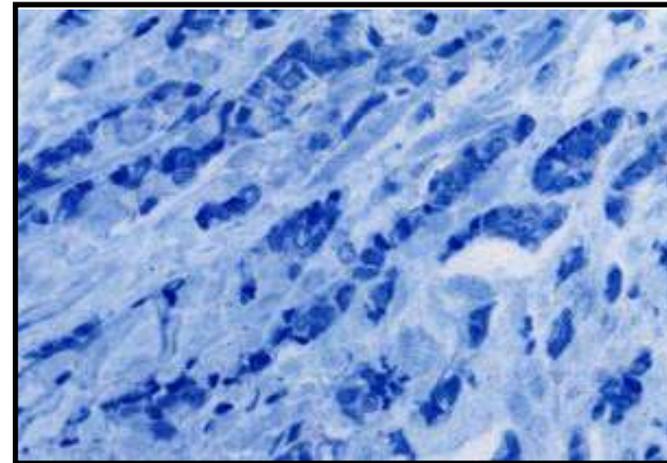
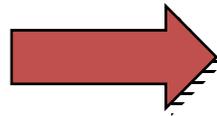
# HER2 scoring: COSA FARE E COSA NON FARE

- Valutare la **PERCENTUALE** e la **INTENSITA'** delle cellule che mostrano una positività di membrana completa
- Una positività **CITOPLASMATICA** non deve essere considerata quando si interpretano i risultati dell'immunoistochimica
- Valutare la colorazione immunoistochimica nella **componente INVASIVA** e non nella eventuale componente *in situ*
- **Le cellule epiteliali NORMALI** dovrebbero mostrare una positività **DEBOLE** di membrana; se si dovesse osservare una positività intensa, il test deve essere ripetuto!

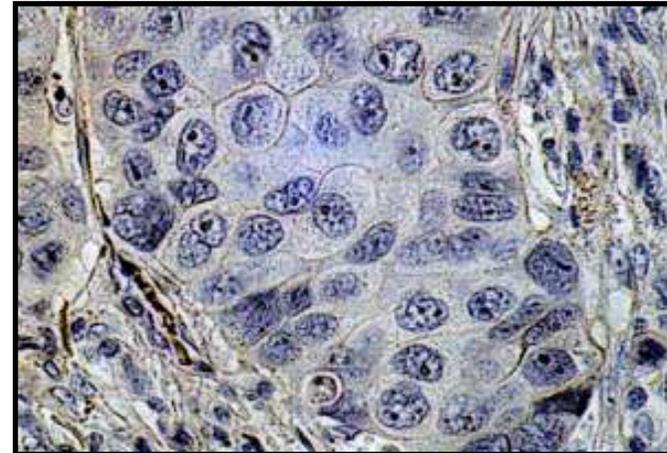
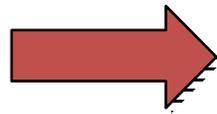
# HER2 - score

**Score: 0**  
**NEGATIVO**

Nessuna espressione



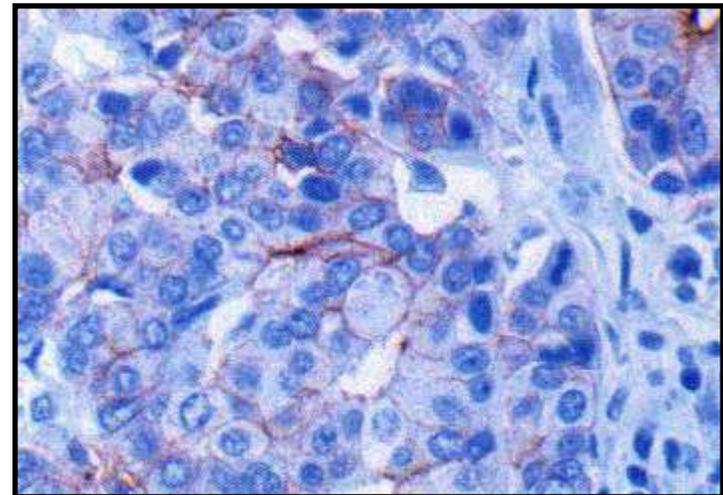
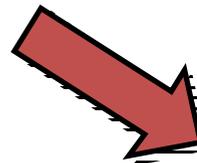
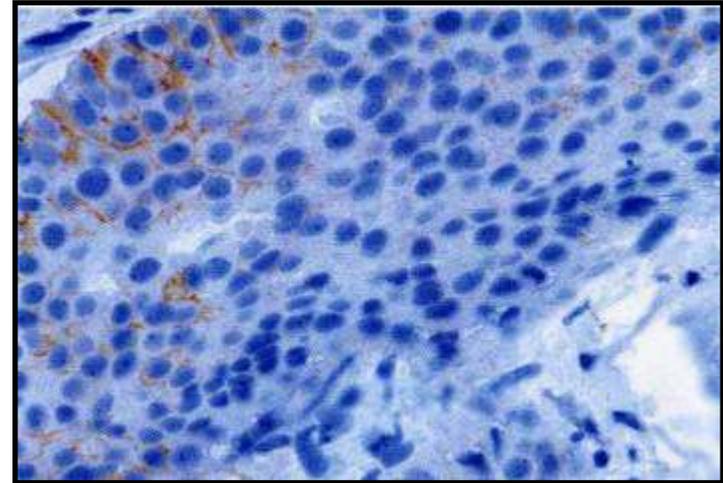
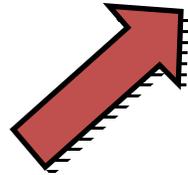
Positività di membrana  
in meno del 10% delle  
cellule tumorali



# HER2 - score

**Score: 1+**  
**NEGATIVO**

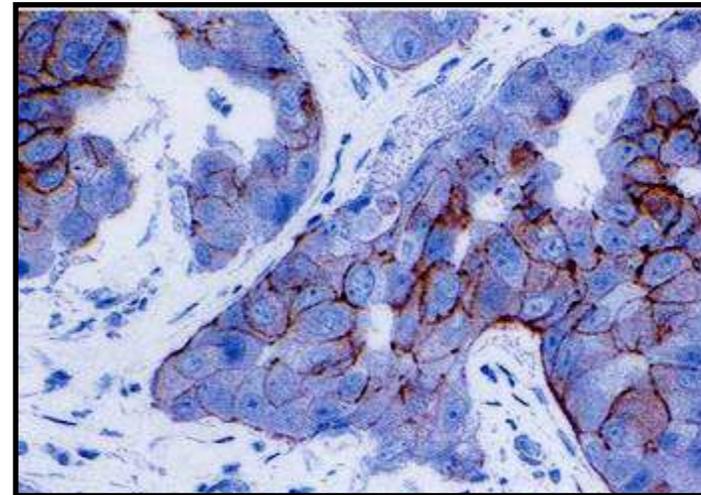
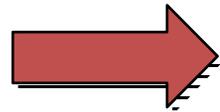
Una debole e incompleta  
positività in >10% delle  
cellule tumorali



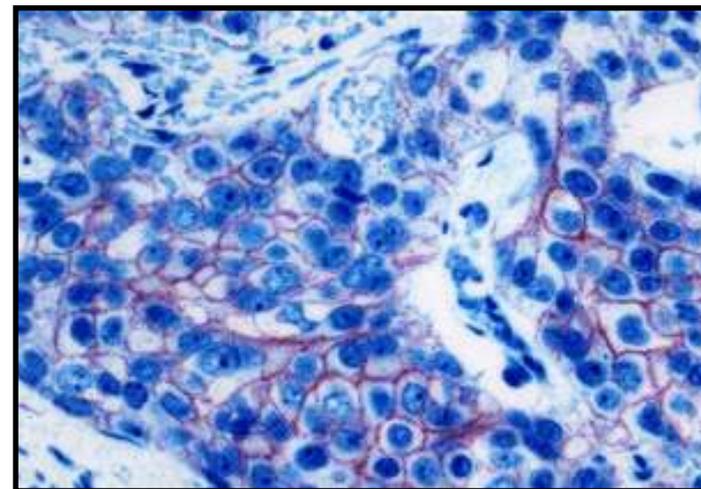
# HER2 - score

## Score: 2+ EQUIVOCO

Una positività di membrana incompleta di intensità da debole a moderata in  $>10\%$  delle cellule tumorali



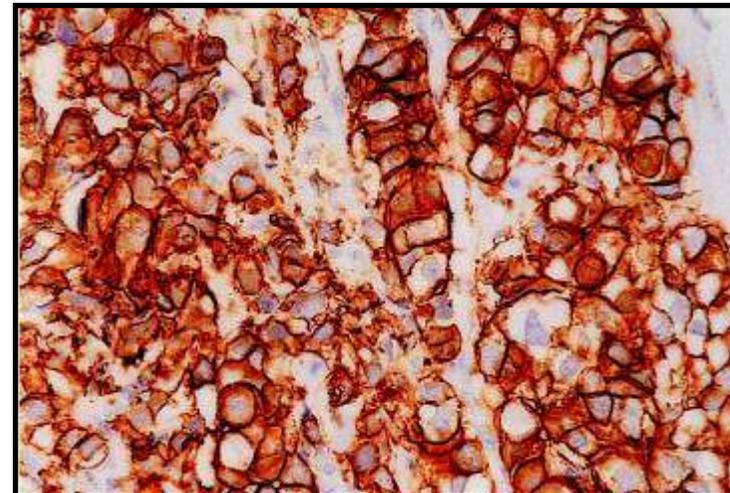
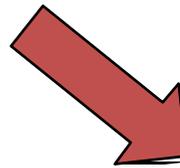
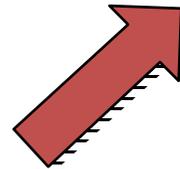
Una positività di membrana completa di intensità da moderata a intensa in  $<10\%$  delle cellule tumorali



# HER2 - score

**Score: 3+**  
**POSITIVO**

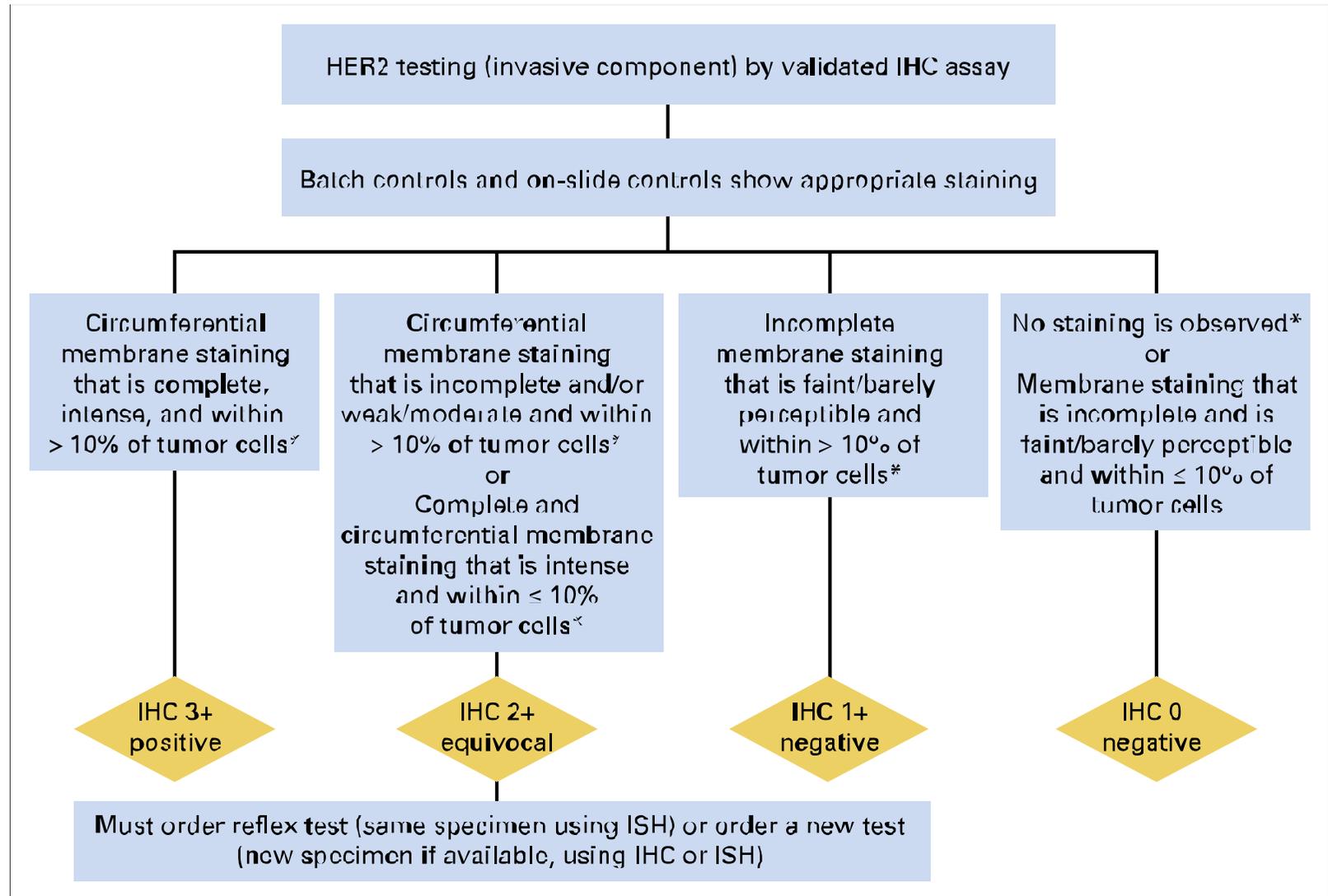
Positività di membrana  
intensa e completa in >10%  
delle cellule tumorali



# HER2 - score

Wolff et al

## Secondo ASCO/CAP 2013



# HER2 - score

## Secondo AIFA/AIOM-SIAPEC

### ~~2010~~ **Score 0**

Assenza di colorazione di membrana (<10% delle cellule) delle cellule di carcinoma invasivo

### **Score 1**

Colorazione di membrana incompleta e di debole intensità presente in qualsiasi percentuale (>10%) delle cellule di carcinoma invasivo

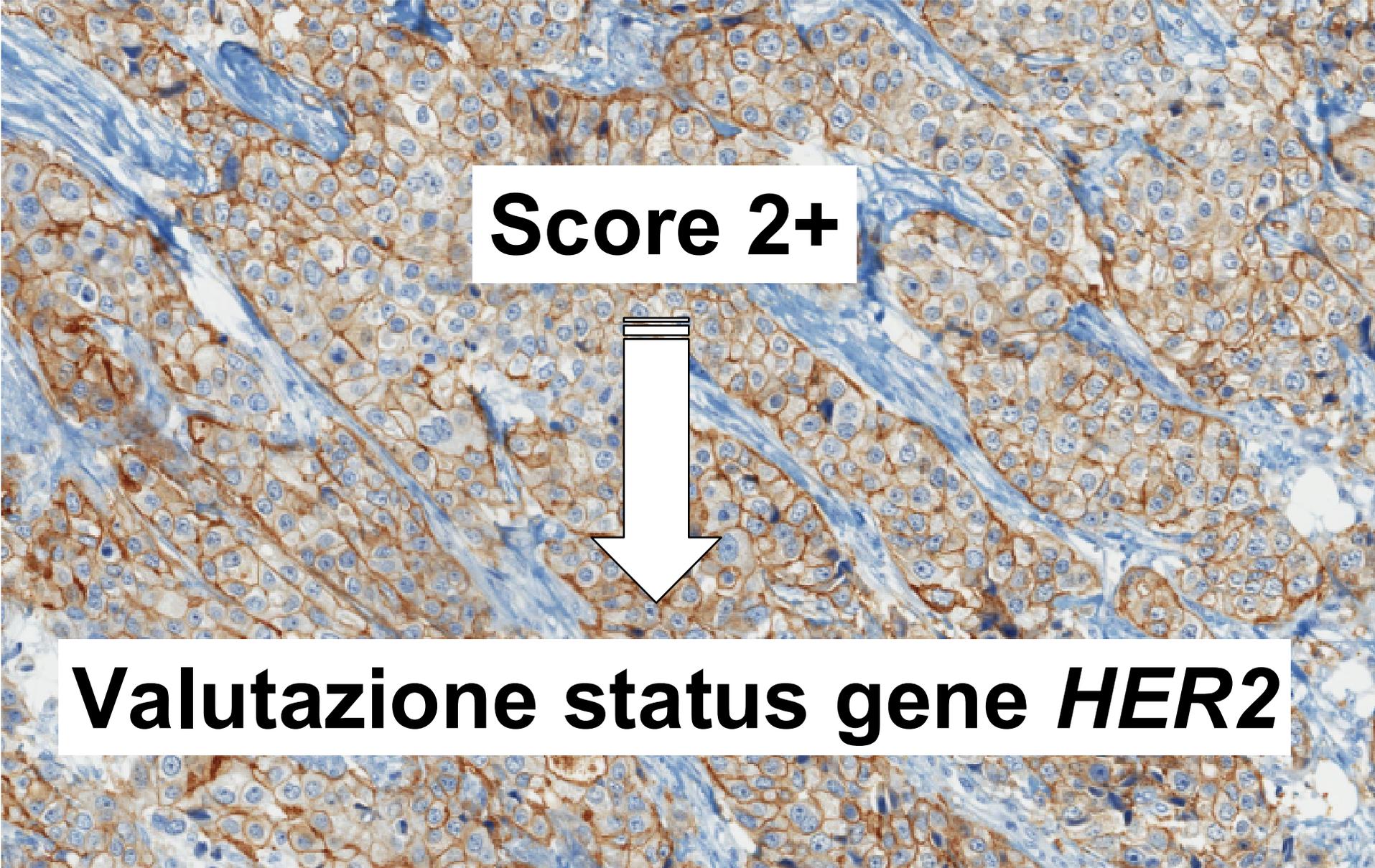
### **Score 2**

-Colorazione di membrana **completa, non uniforme oppure di debole intensità** ma comunque con evidente distribuzione circonferenziale, presente in **almeno il 10%** delle cellule di carcinoma invasivo

-Colorazione di membrana **completa e intensa** ma presente in **<30%** delle cellule di carcinoma invasivo

### **Score 3**

Colorazione di membrana **completa, uniforme e intensa** in **almeno il 30%** delle cellule di carcinoma invasivo



**Score 2+**

**Valutazione status gene *HER2***