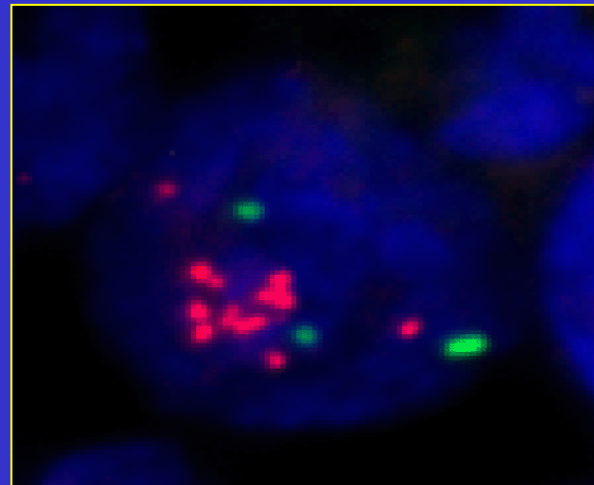
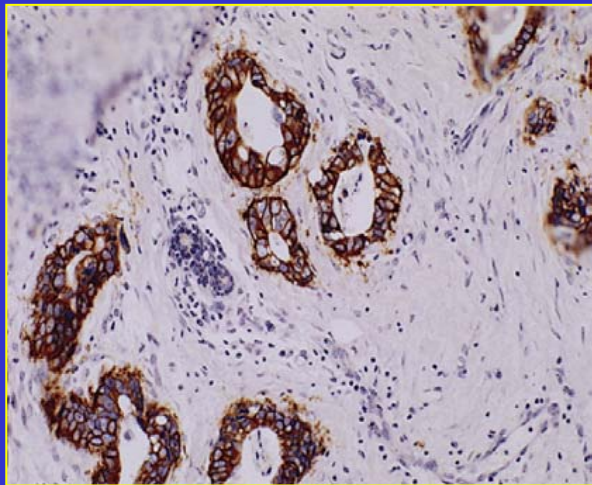


Molecular Pathology applied to Breast Cancer « The HER2 revolution? »

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*Laboratory CMP, Brussels - www.labocmp.be
with the contribution of Dako & Roche Pharmaceutica*

Terminology « HER-2 »

- **Gene:** oncogene HER-2/neu (isolated from rat neuroblastoma) also named *c-erbB-2* (human DNA)
- **Coded onco-protein:** P185^{HER2}
- **Protein function:** Human Epidermal growth factor **Receptor-2** (EGFR family)

The epidermal growth factor receptor family

GENE

HER1
(*c-erbB-1*)

HER2
(*c-erbB-2*)

HER3
(*c-erbB-3*)

HER4
(*c-erbB-4*)

LIGAND

EGF
TGF- α
Beta cellulin
Heparin-binding
Growth Factor
Amphiregulin
Epiregulin

?

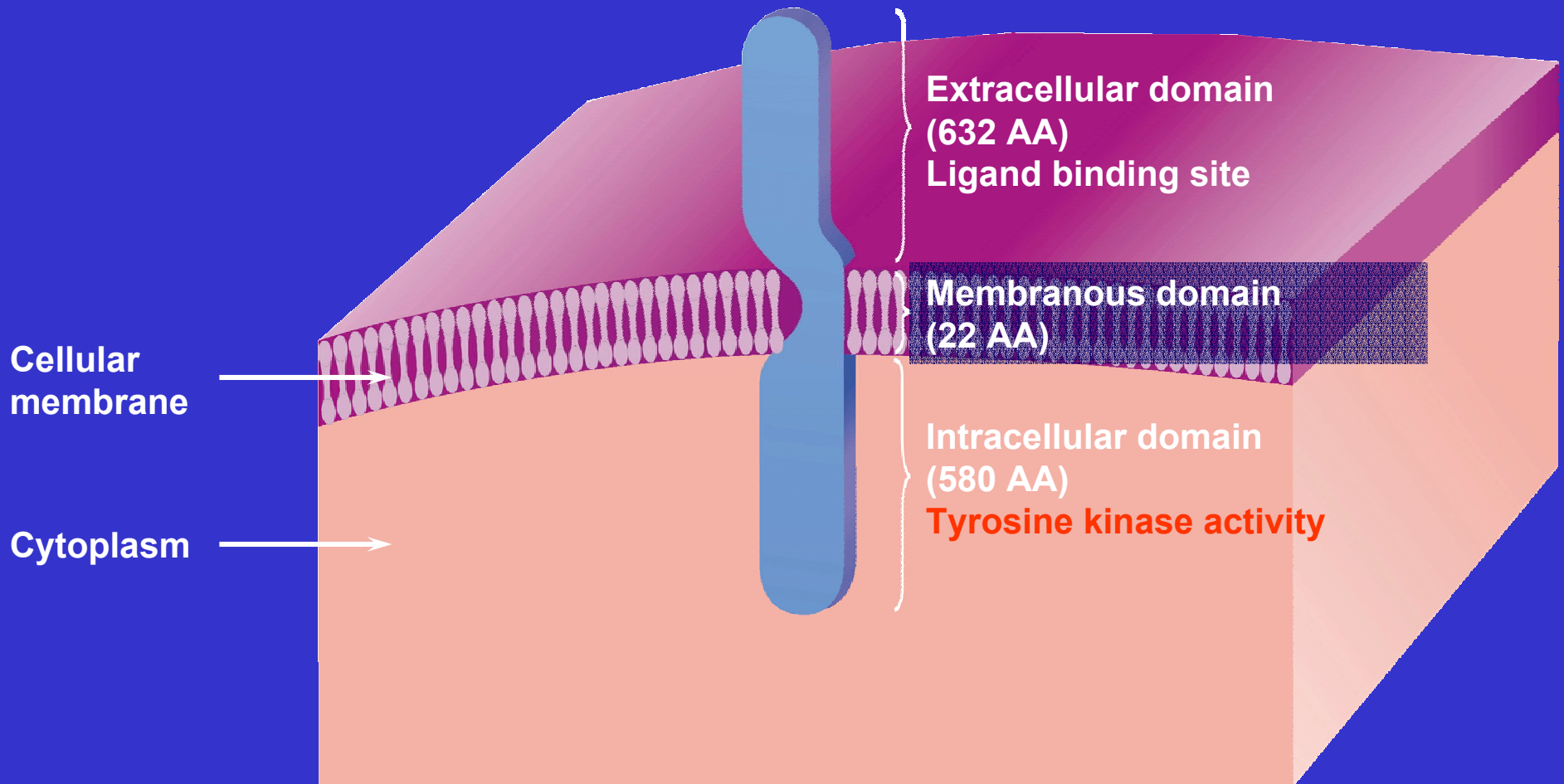
Neuregulins

Neuregulins

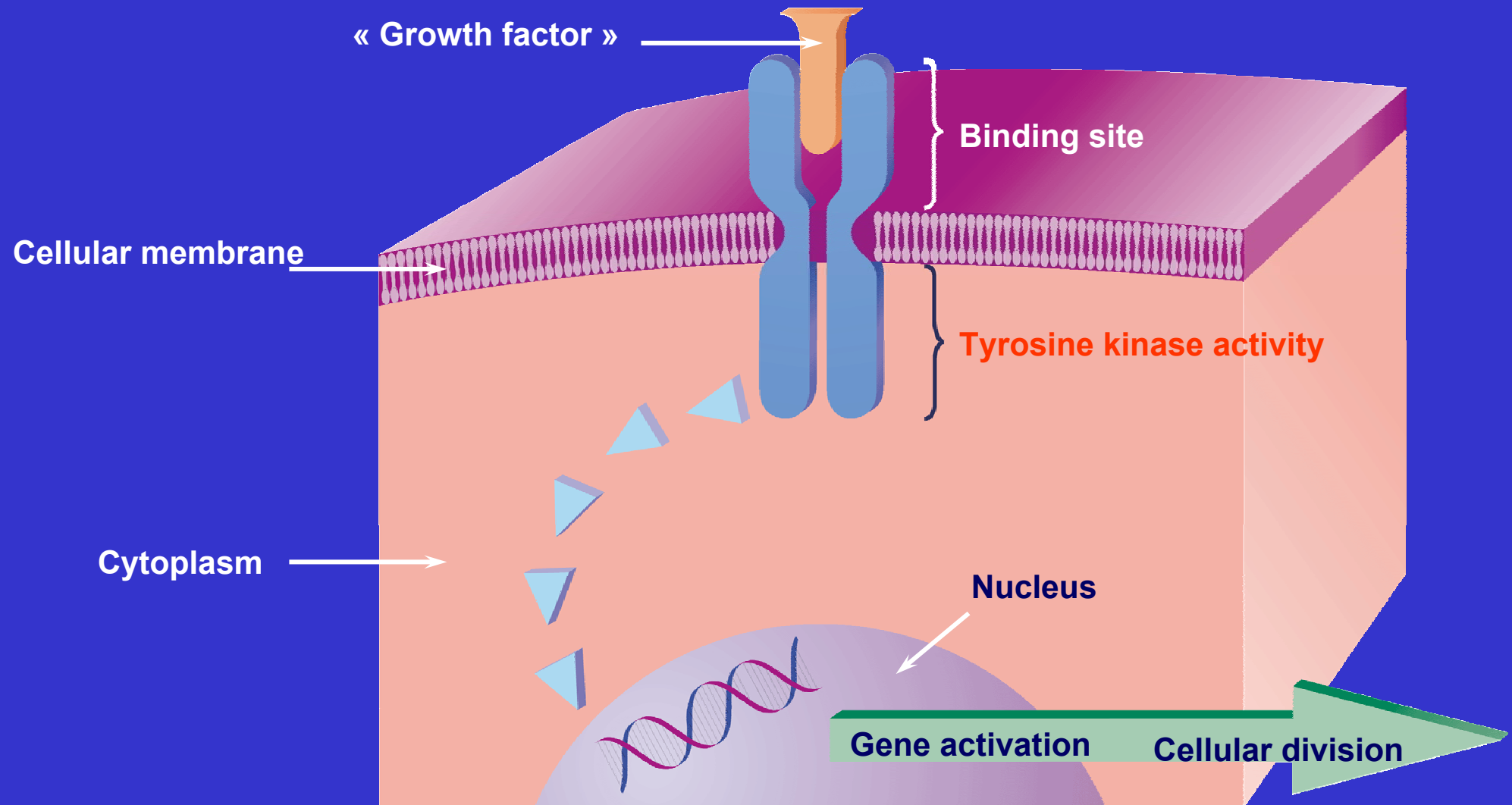
EGF = Epidermal Growth Factor

TGF- α = Transforming Growth Factor Alpha

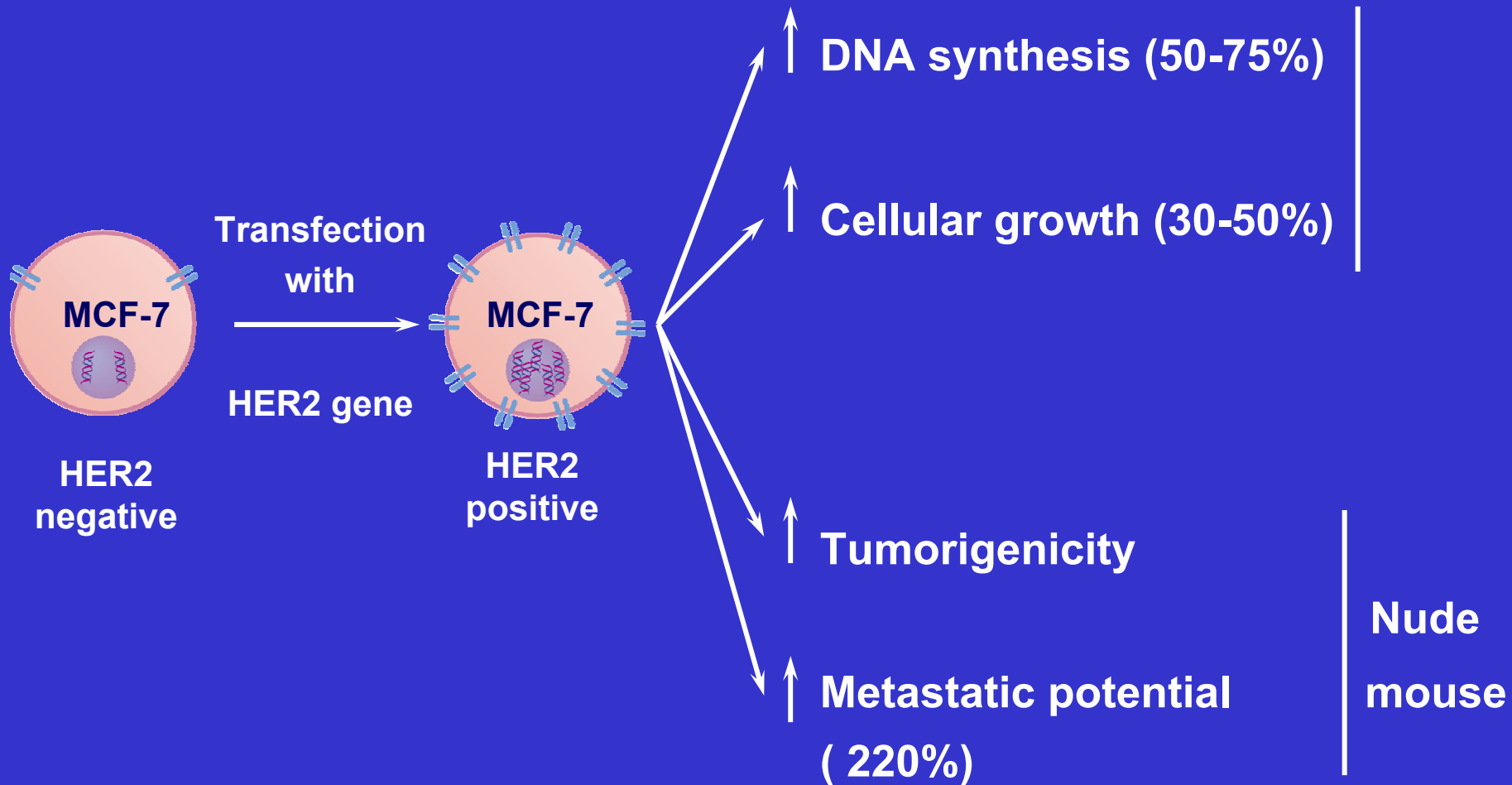
Structure of monomeric oncoprotein P185^{HER2}



Cellular activity of the dimeric P185^{HER2} oncoprotein



Consequences of HER2 amplification in human breast cancer cell lines



Relation between HER2 and Breast Cancer

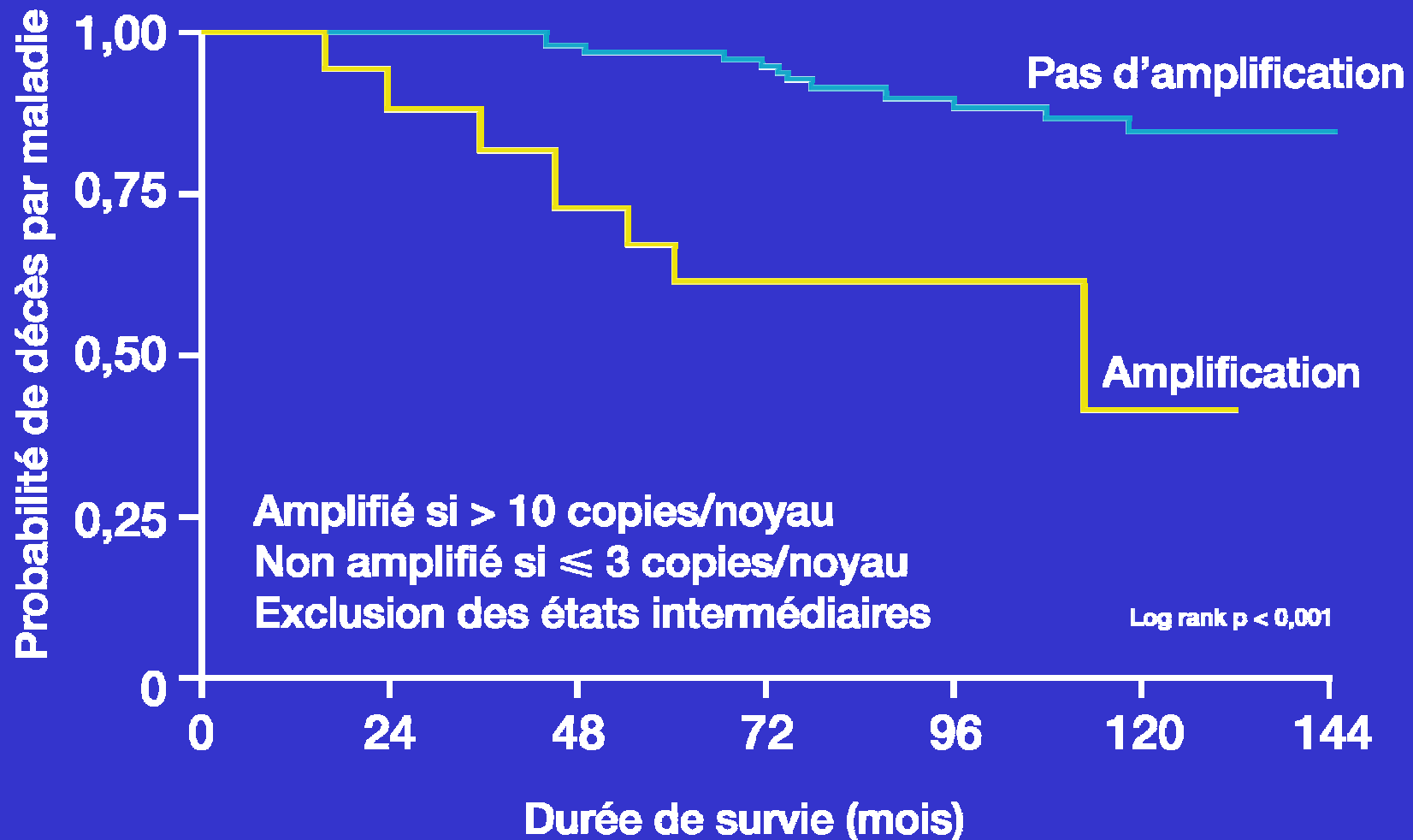
- Oncogene HER2 is amplified and/or P185^{HER2} is overexpressed in 25 to 30% of IDC and in 60 to 70% of DCIS but rarely in non malignant lesions.
- Oncogene HER2 is amplified and/or P185^{HER2} is overexpressed in 30% of metastatic breast cancer

Relation between HER2 and Breast Cancer

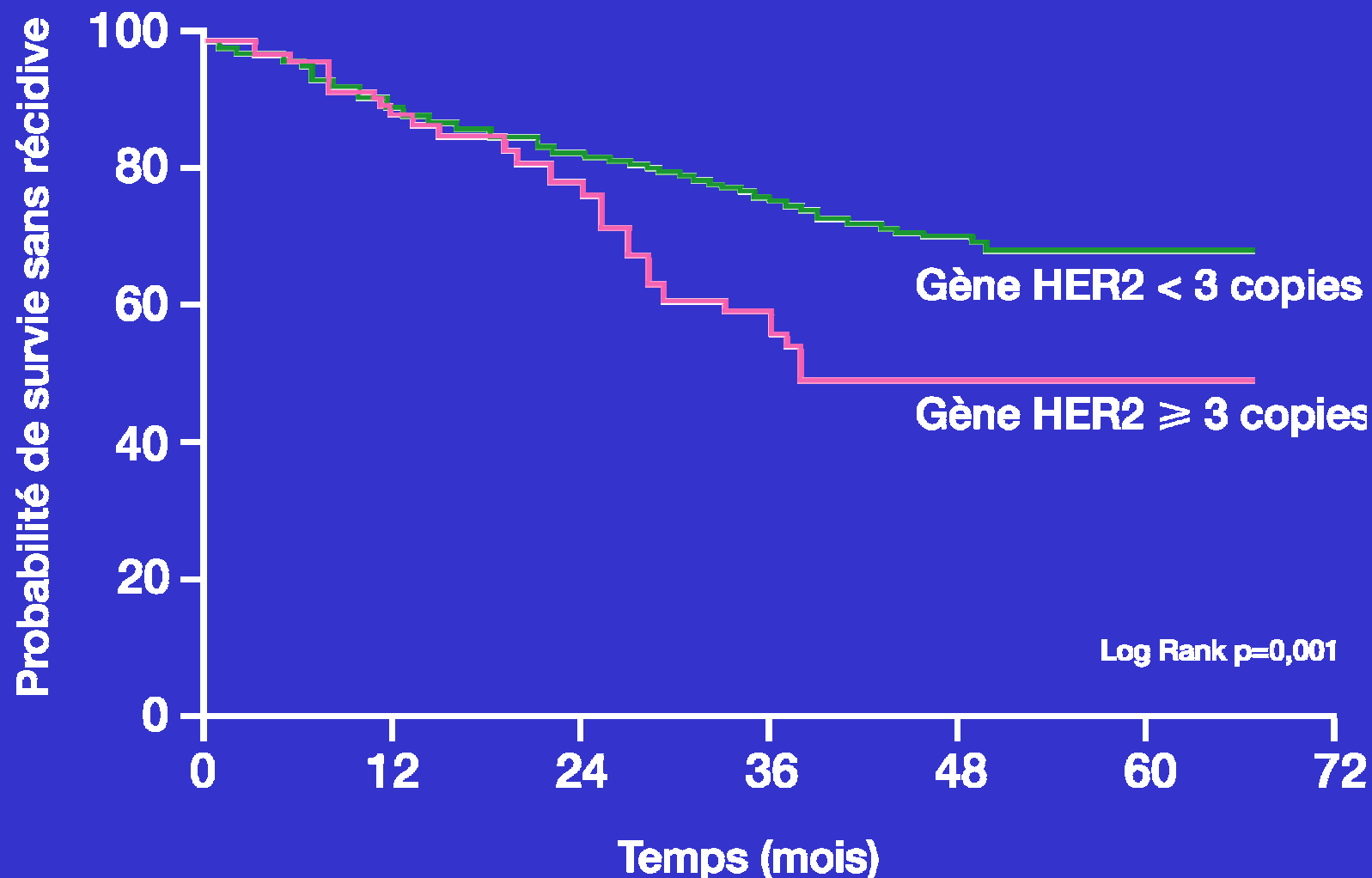
Prognostic factor	Univariate		Multivariate	
	Survival	Recurrence	Survival	Recurrence
nb N+	0,0001	0,0002	0,0003	0,001
HER2	0,0011	<0,0001	0,02	0,001
RP	0,05	0,05	N/A	N/A
Tumor size	0,06	0,06	N/A	N/A
RE	0,15	0,10	0,03	N/A

n = 86 patients N+/Southern blot assay/median follow-up 46 months

Overall survival of N- breast cancer patients and HER2 status



Disease free survival of N+ breast cancer patients and HER2 status



HER2 – A predictive factor for the response to hormone-chemotherapy?

Hormonotherapy

Undetermined

HER2 positivity might be significant for resistance

Anthracyclins

Possible

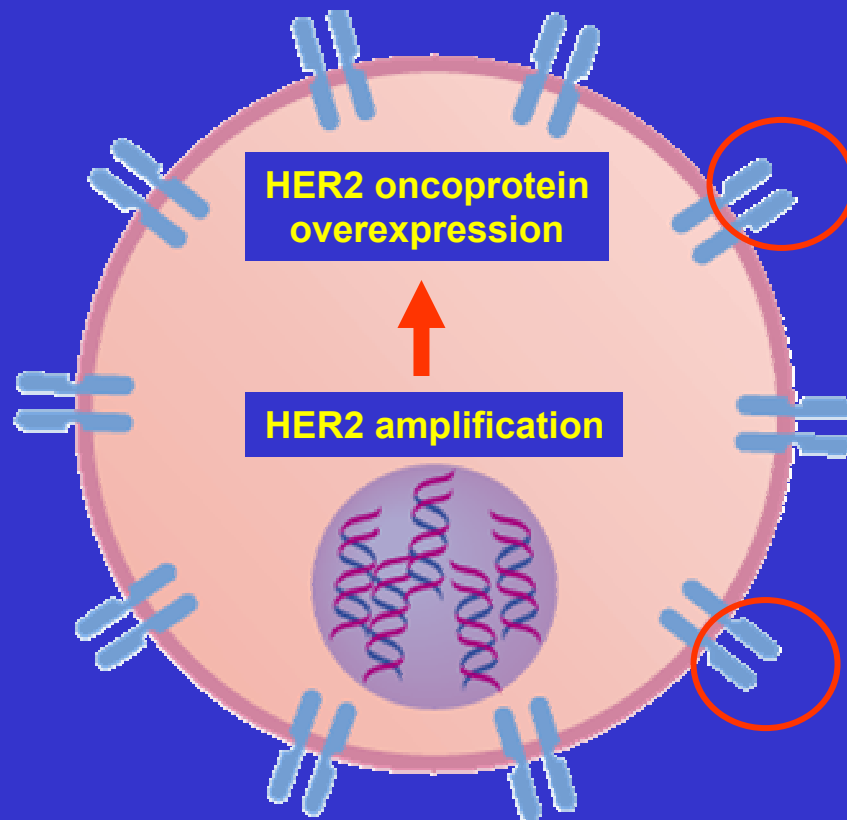
HER2 positivity might indicate response to high dose anthracyclins chemotherapy

CMF

Undetermined

HER2 positivity might be significant for resistance

HER2 a potential target for immunotherapy of breast cancer patients



Trastuzumab (Herceptin)
4D5 humanized monoclonal antibody directed against the extracellular domain of HER2 protein

Mechanism of action of Trastuzumab

- Non clinical pharmacology studies suggest trastuzumab may **antagonize** the function of the growth properties of the HER2 system, enlist **immune cells to attack and kill the tumor target**

Clinical trials: effects of Trastuzumab treatment

- **Trastuzumab will be an important new treatment option for women with HER2-overexpressing metastatic breast cancer**
 - **NSABP B-31 protocol initiated to test the advantage of adding Herceptin to adjuvant chemotherapy in stage II breast cancer with HER2 overexpression**

Shak S et al. Semin Oncol 26: 71-77, 1999 – Vogel C et al. J Clin Oncol 20: 719-26, 2002

Paik S et al. J Natl Cancer Inst 94: 852-4; 2002

Clinical trials: Protocol BIG 01-01/BO16348B

- **HERA: A randomized three-arm multicentre comparison of 1 year and 2 years Herceptin versus no-Herceptin in women with HER2-positive primary breast cancer who have completed adjuvant chemotherapy**
- **Started september 2002. Total enrollment 3192 patients**

Method of detection of HER2 overexpression

Method of detection	Target HER2 molecule	Mechanisms
IHC	P185^{HER2}	Overexpression
FISH	HER2 oncogene	Amplification
ELISA	P185^{HER2}	Circulating HER2 receptor
Western Blot	P185^{HER2}	Overexpression
Northern Blot	HER2 RNAm	Overexpression
Southern, slot blots	HER2 oncogene	Amplification
RT-PCR	HER2 oncogene	Amplification

IHC : Immunohistochimie - FISH : Fluorescence *in situ* hybridation - ELISA : Enzyme-linked immunosorbent assay

IHC Interpretation HER2 over-expression

Staining Pattern HercepTest

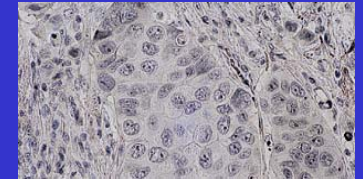
Score

Assessment & Example

No staining is observed or membrane staining is observed in less than 10% of the tumour cells.

0

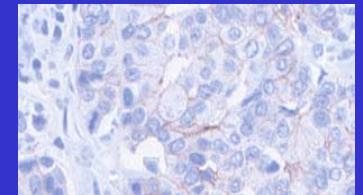
Negative



A faint/barely perceptible membrane staining is seen in more than 10% of the tumour cells. The cells are only stained in part of their membrane.

1+

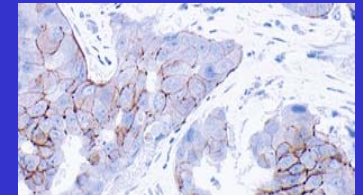
Negative



A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells.

2+

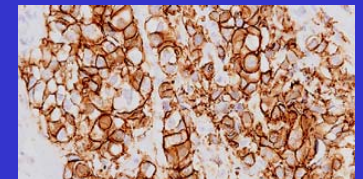
Weak +



A strong complete membrane staining is observed in more than 10% of the tumour cells.

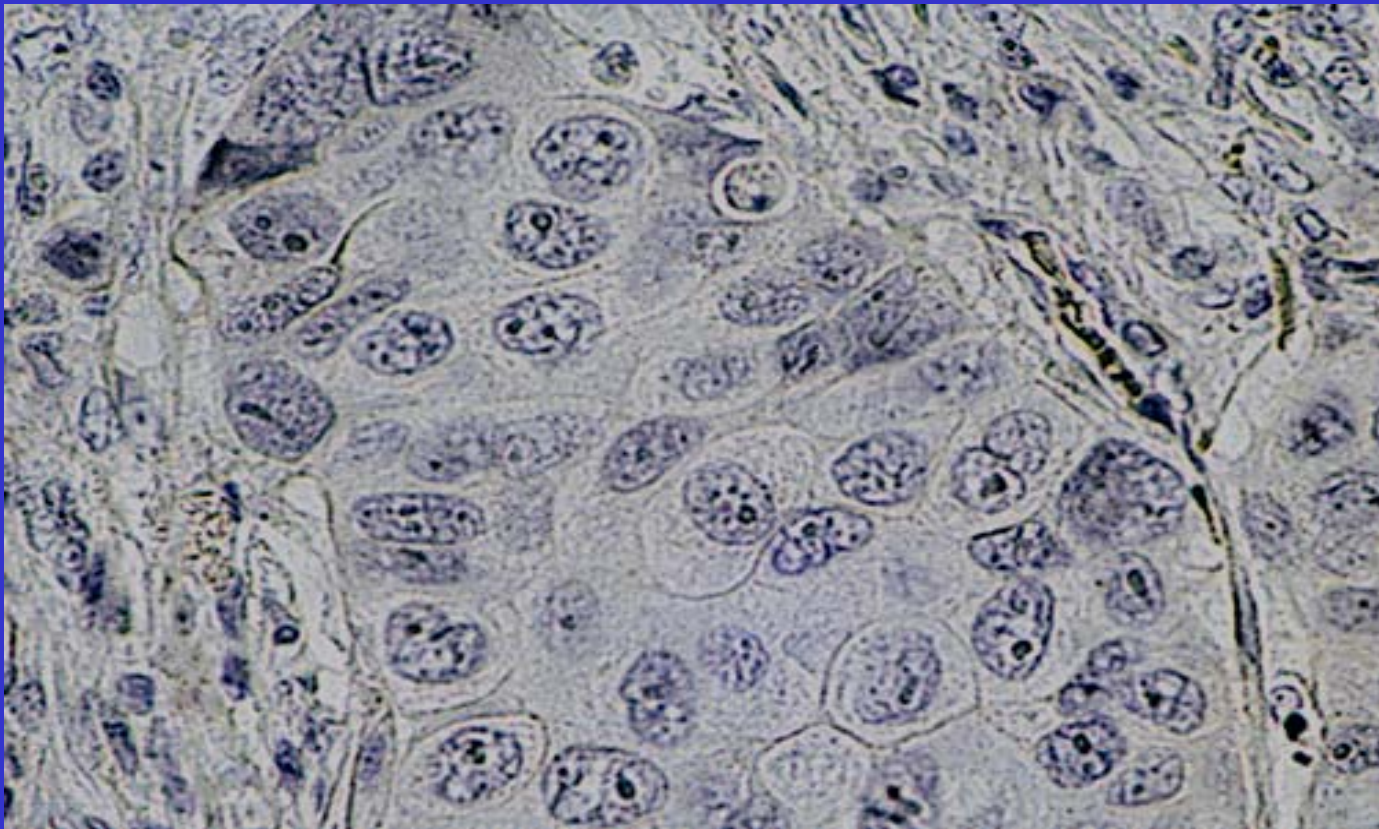
3+

Strong +



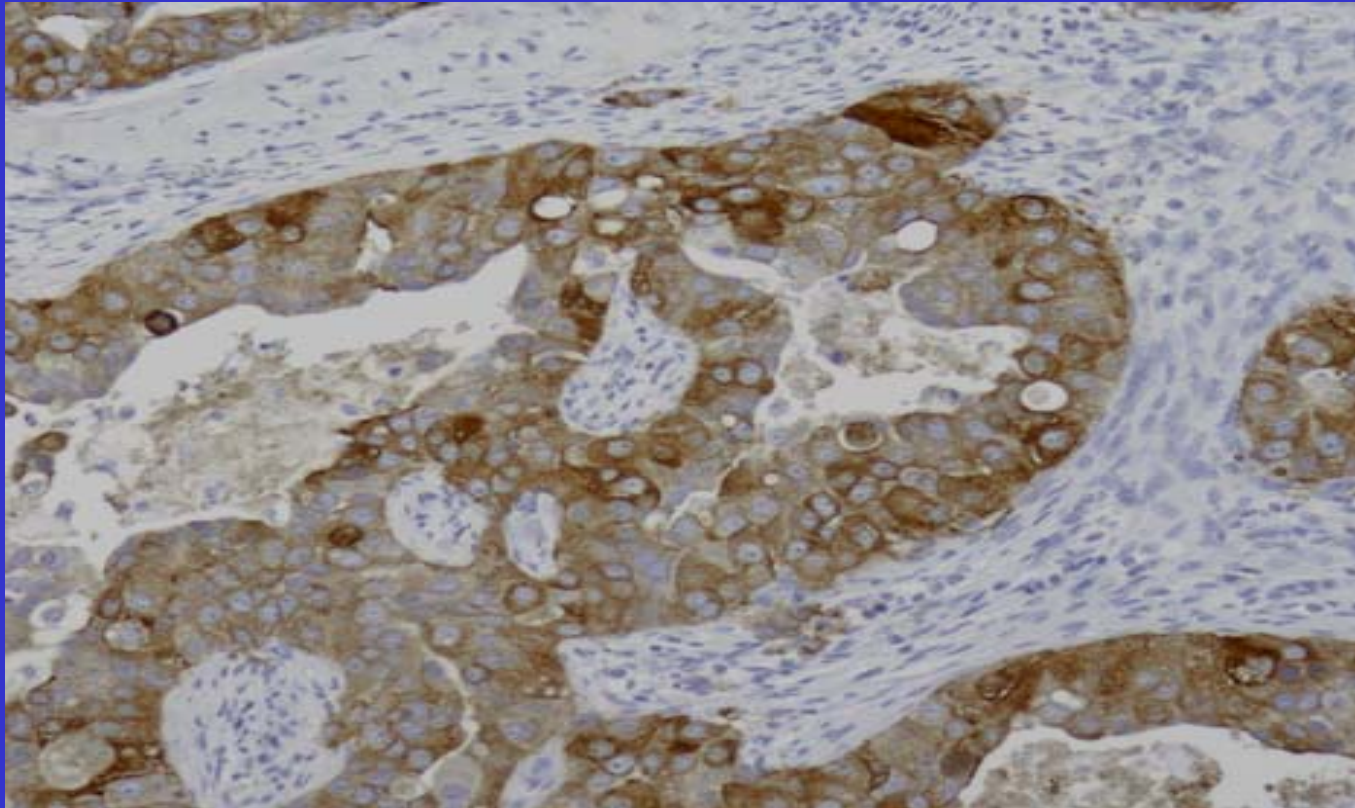
IHC “0” Score (Negative)

No staining is observed or membrane staining is observed in less than 10% of the tumour cells.



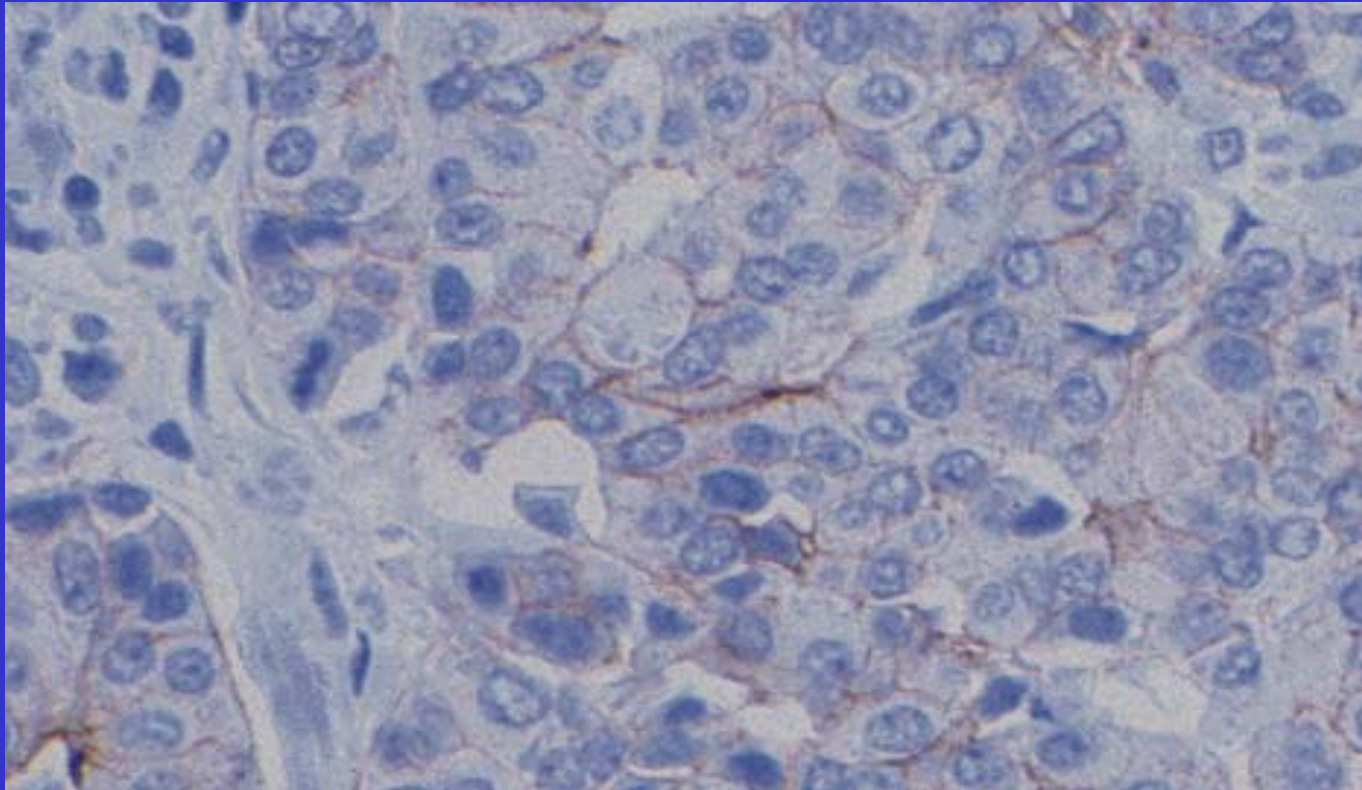
IHC “0” Score (Negative)

- ◆ Diffuse non specific homogenous stain specifically confined to the cytoplasm



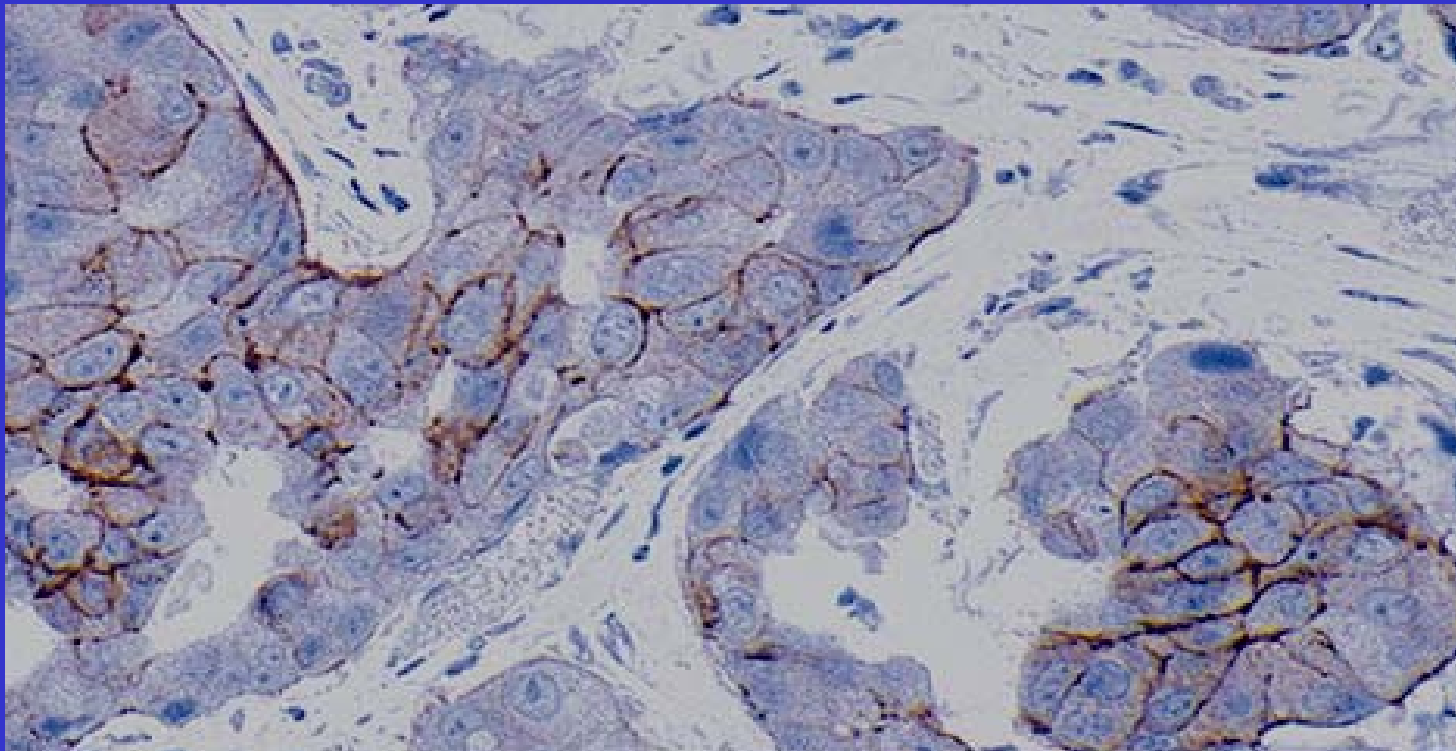
IHC “1+” Score (Negative)

A faint / barely perceptible membrane staining is seen in more than 10% of the tumour cells. The cells are only stained in part of their membrane.



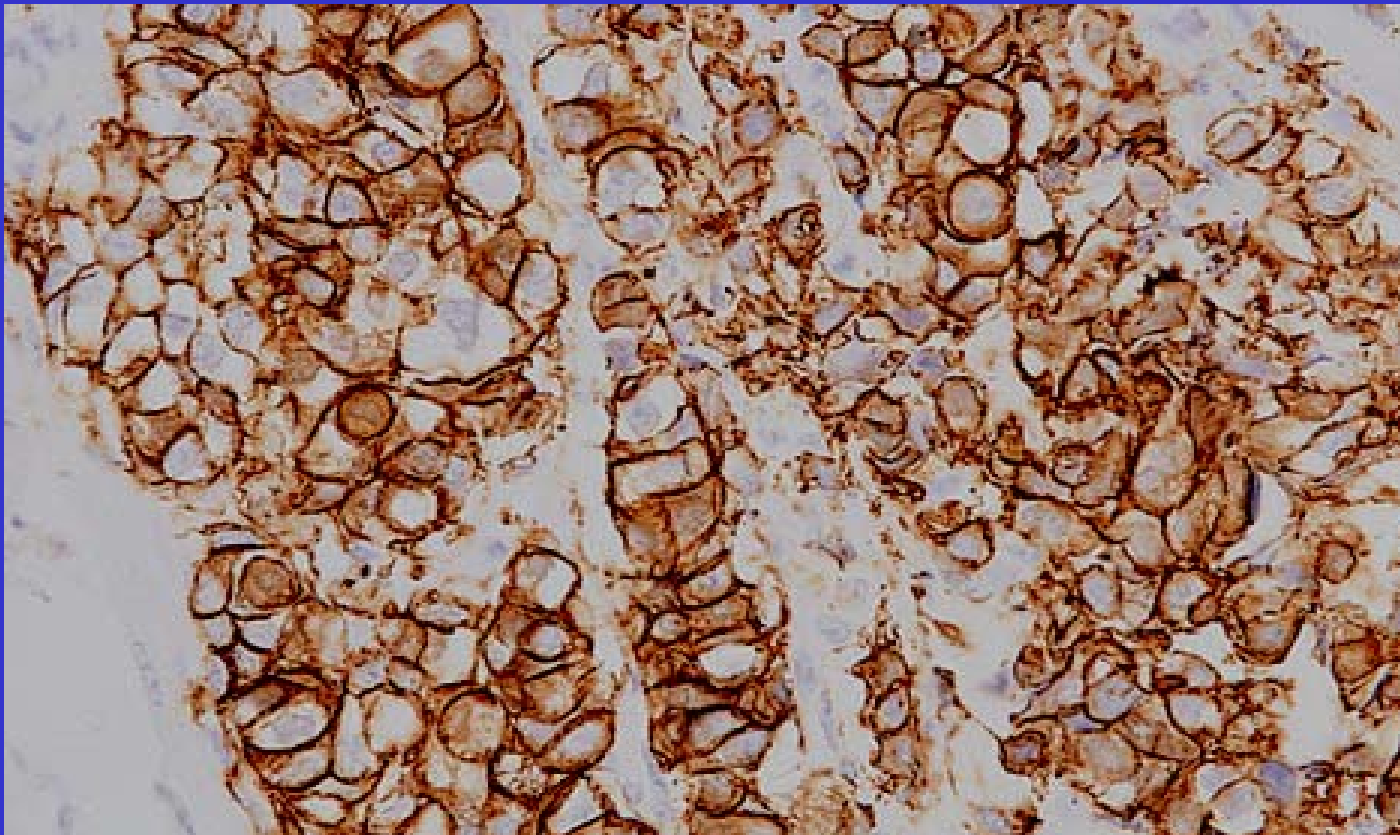
IHC “2+” Score (Positive)

A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells.



IHC “3+” Score (Positive)

A strong complete membrane staining is observed in more than 10% of the tumour cells.



Eligibility for anti-HER2 according to IHC

Score IHC

Eligibility

anti-HER2

0

non eligible

1+

non eligible

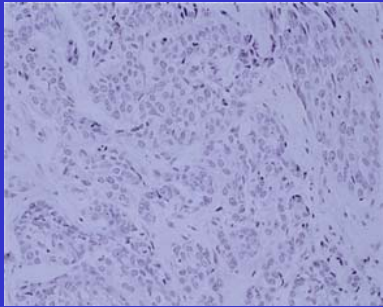
2+

eligible

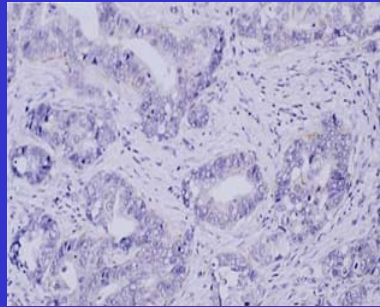
3+

eligible

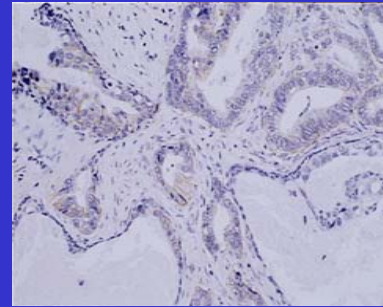
HER2 FISH or IHC ?



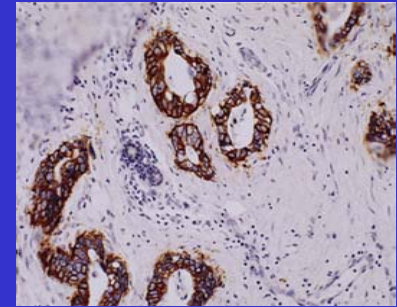
IHC 0



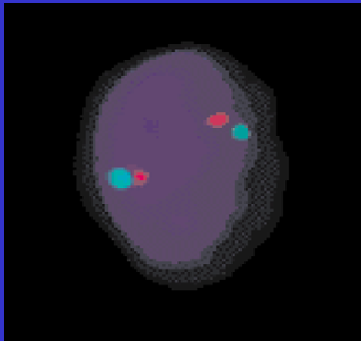
IHC 1+



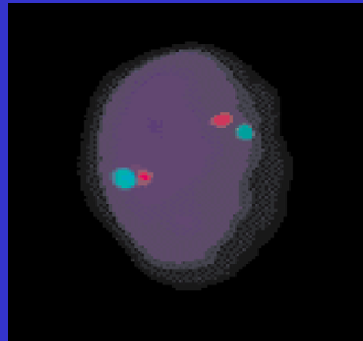
IHC 2+



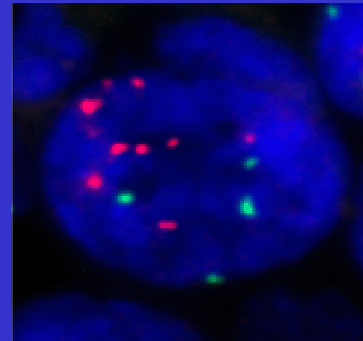
IHC 3+



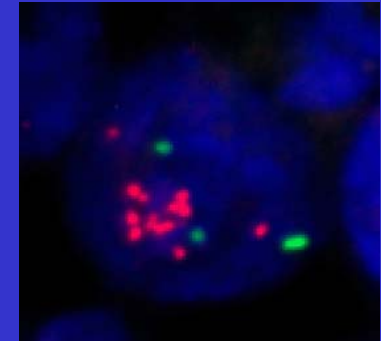
**No
amplification**



**No
amplification**



**Weak
amplification**



**Strong
amplification**

HER2 FISH versus IHC ?

- **Concordance between both methods varies from 80.3% to 92.4% depending on studies, antibodies,...**
- **Sensitive standardized IHC remains the first choice but all specimens scored 2+ should be evaluated by FISH. The results of both assays should be considered before recommending anti-HER2 therapy**

Paik S et al. J Natl Cancer Inst - Perez E et al. Mayo Clin Proc - Kobayashi M et al. Hum pathol 2002

Tsuda H et al. Cancer 2001

Perspectives: HER2 positivity in other cancers

Primary cancers (n)	P185 ^{HER2} overexpression (%)
Ovary (n = 73)	32%
Stomach (n = 459)	12 - 55%
Lung NSC (n = 207)	27 - 56%
Sarcoma (n = 94)	37%
Bladder (n = 141)	36%
Oesophagus (n = 25)	60 - 73%
Salivary glands (n = 27)	32 - 62%

Conclusions & Perspectives

- Immunotherapy of human malignancies is beginning to realize the promise expected more than 20 years ago.
- Anti-HER2/neu trastuzumab should be regarded as the first efficient Mab treatment in Phase II-III clinical trials of metastatic breast cancer patients
 - Antibody-based strategies are prone to develop in the very next future

Conclusions & Perspectives

- The incidence of MolPath assessments in routine pathology should dramatically increase soon (CD20 & malignant B cells, EGFR & colon cancer, HER2 in non-breast cancer,...)
- The pathologists should be aware of the analysis procedures and consequences for clinical applications
- These developments pose critical problems in terms of financing and quality assurance