



# ¿Son iguales todos los carcinomas de mama HER2+?

Federico Rojo  
IIS-Fundación Jiménez Díaz  
IMIM-Hospital del Mar

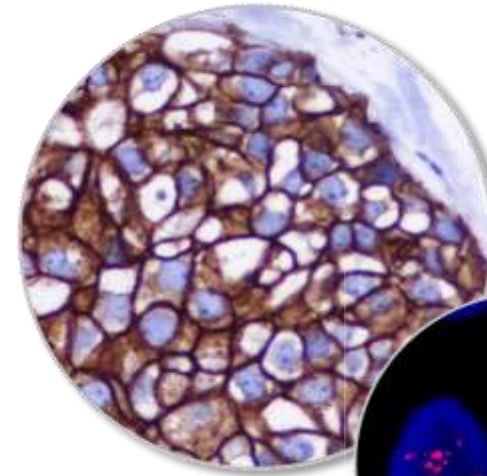
Curso Corto de Patología Mamaria  
Nuevos Fenotipos del Cáncer de Mama  
¿Nuevos Problemas para el Patólogo?

XXV Congreso de la Sociedad Española de Anatomía Patológica y  
División Española de la Academia Internacional de Patología (SEAP-IAP)

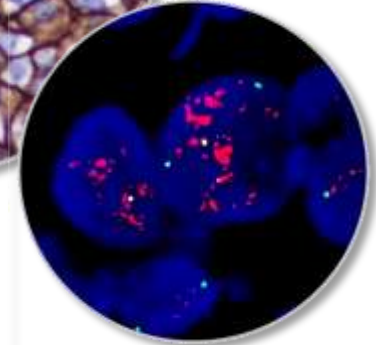
Zaragoza, Mayo 2011

# Cuatro carcinomas de mama con sobreexpresión de HER2

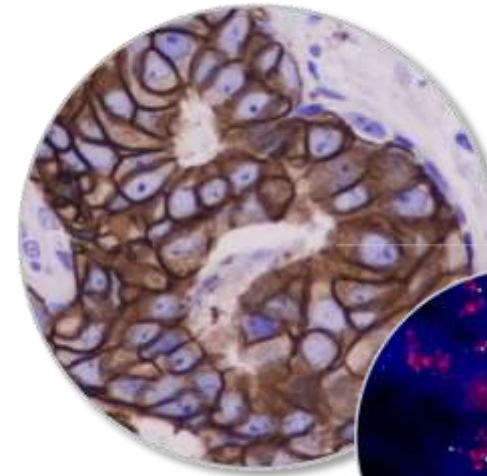
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CDI 20mm, pN0  
ER+/PR-  
HER2 3+, ratio 3  
FACx6  
Herceptin 1 año  
No recidiva tras 8 años



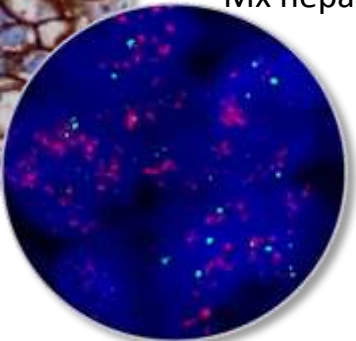
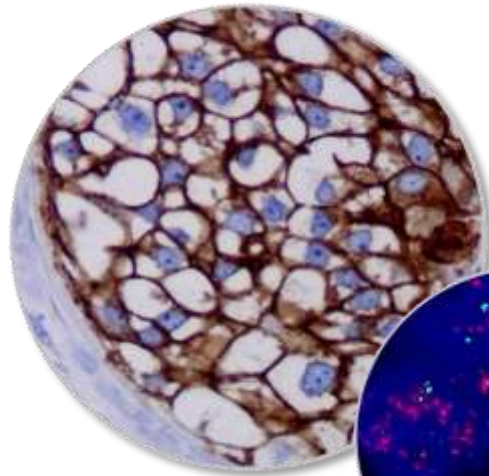
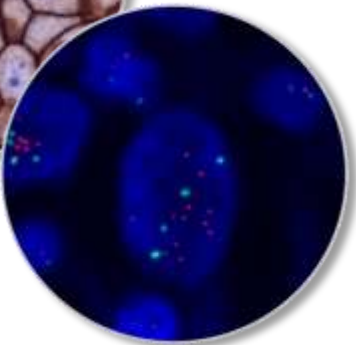
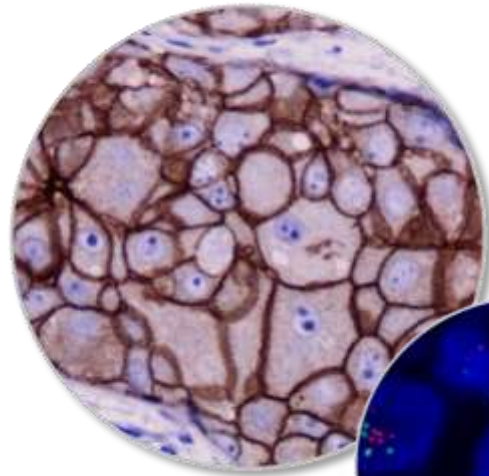
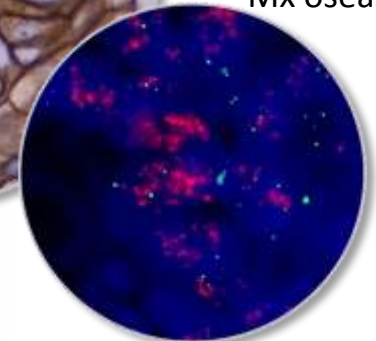
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FACx6  
Herceptin 1 año  
Recidiva local a 4 años



52 años  
CDI 22mm, pN0  
ER-/PR-  
HER2 3+, ratio 7  
FACx6  
Herceptin 1 año  
Mx hepáticas a 3 años



49 años  
CDI 18mm, pN0  
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Mx óseas a 4 años



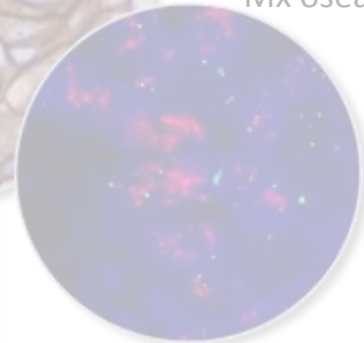
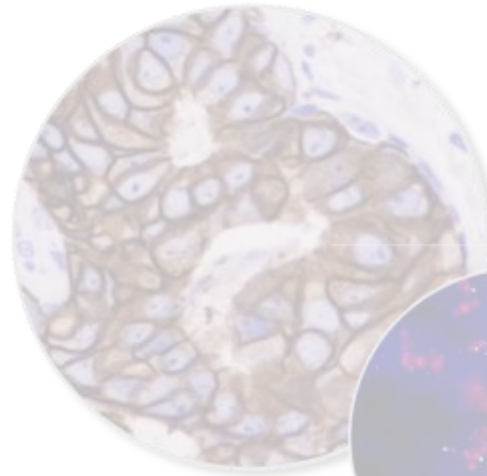
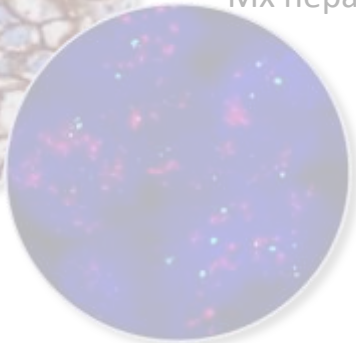
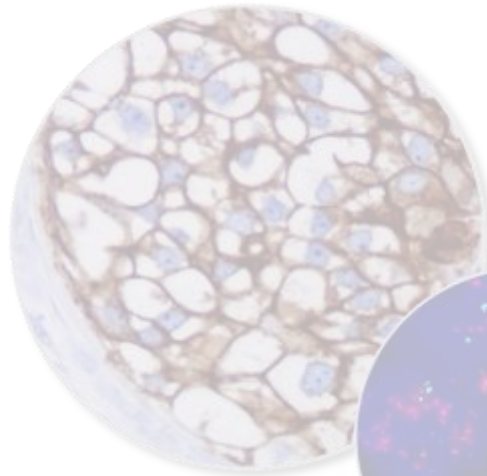
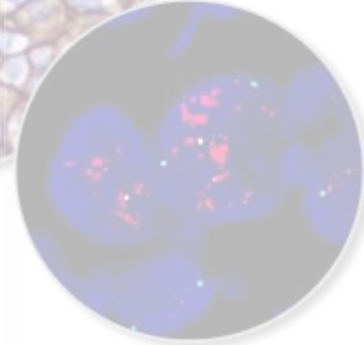
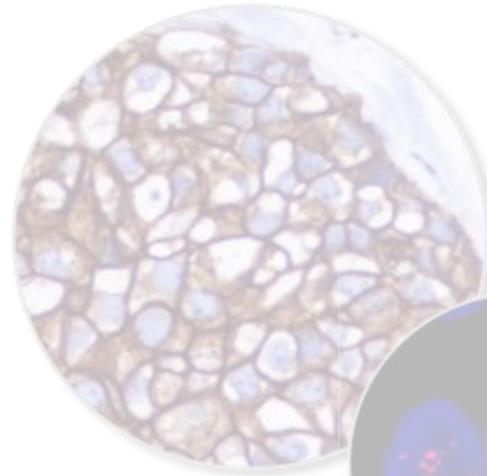
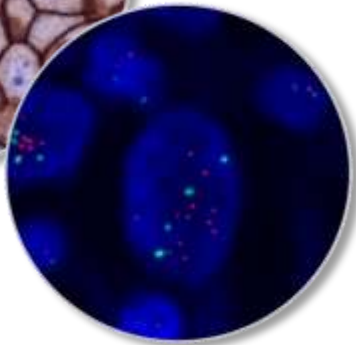
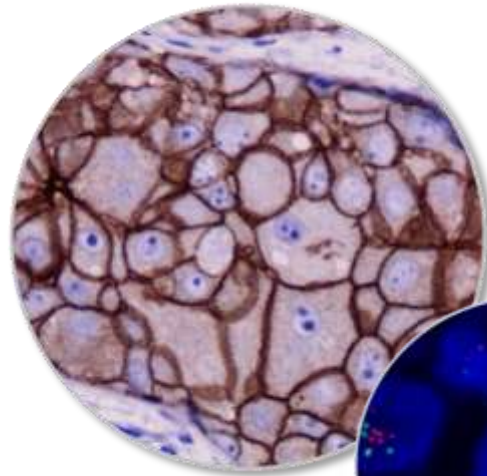
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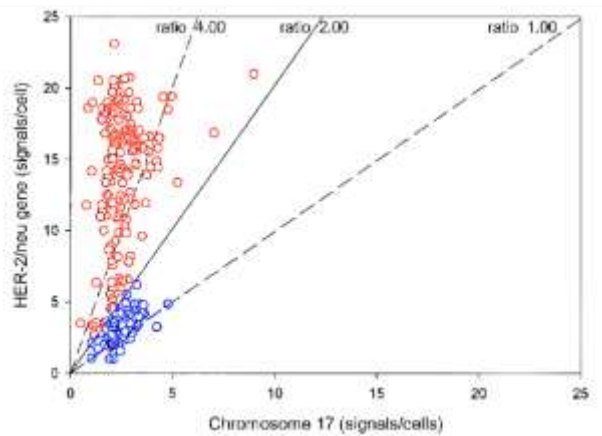
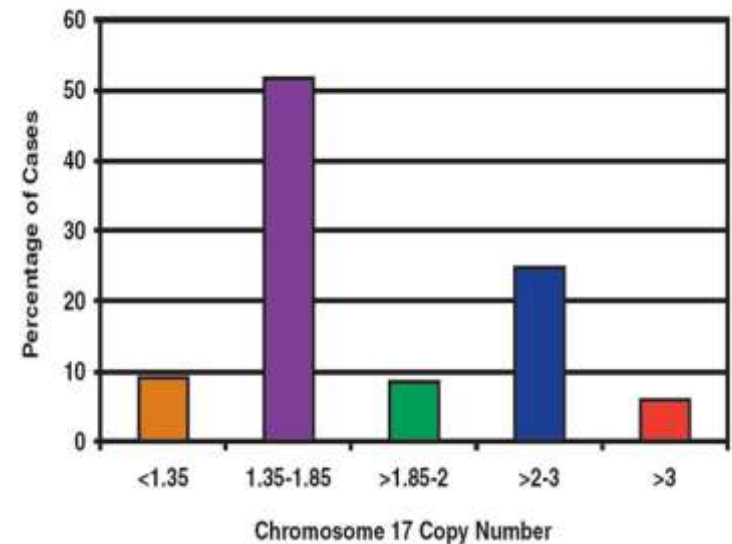
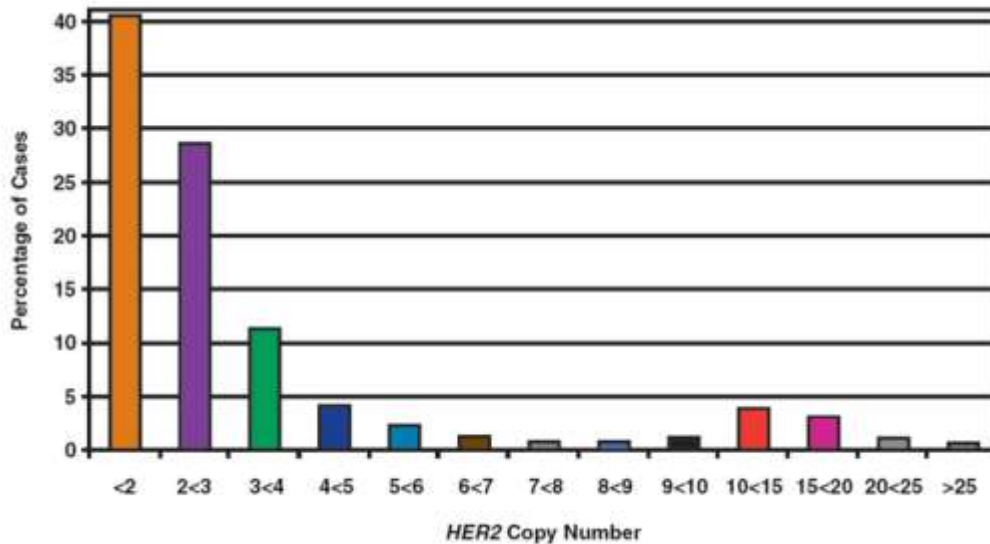
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# Distribution of HER2 gene copy number and chromosome 17 number in breast cancer

n=1711



Mean <i>HER2</i> Copy Number	Percentage of Total Population	Percentage of Cases With <i>HER2</i> Amplification
<2	40.44	0.00
2 to <3	28.52	3.28
3 to <4	11.40	16.41
4 to <5	4.21	50.00
5 to <6	2.34	67.50
6 to <7	1.29	77.27
≥7	11.80	Virtually 100.00

NA, current guidelines require chromosome 17 testing in this population, which excludes the potential for misdiagnosis.

# HER2 gene copy number does not predict response to trastuzumab: the N9831 adjuvant trial (n=1888)

## Criteria for Classifying Each Specimen

### HER2

- Amplified *HER2*: > 10 *HER2* signals in > 40% of invasive nuclei
- Small clone of amplified *HER2*: > 10 *HER2* signals in > 5 and < 40% of invasive nuclei
- Duplicated *HER2*: having an *HER2*/CEP17 ratio > 1.30, but not amplified *HER2*
- Deleted *HER2* (-*HER2*): having an *HER2*/CEP17 ratio < 0.80

### CEP17

- Polysomic 17 (+17; p17):  $\geq 3$  CEP17 copies in > 30% of invasive nuclei
- Monosomic 17 (-17; m17): 1 CEP17 copy in > 60% of invasive nuclei

The final interpretation combined the *HER2* and CEP17 results as follows:

NACA: Normal for all chromosome 17 anomalies (*HER2*/CEP17 ratio > 0.80 and < 1.30, < 30% nuclei with  $\geq 3$  CEP17 signals, < 60% nuclei with 1 CEP17 signal).

Normal *HER2*, -17: One CEP17 copy in > 60% of invasive nuclei and two *HER2* copies

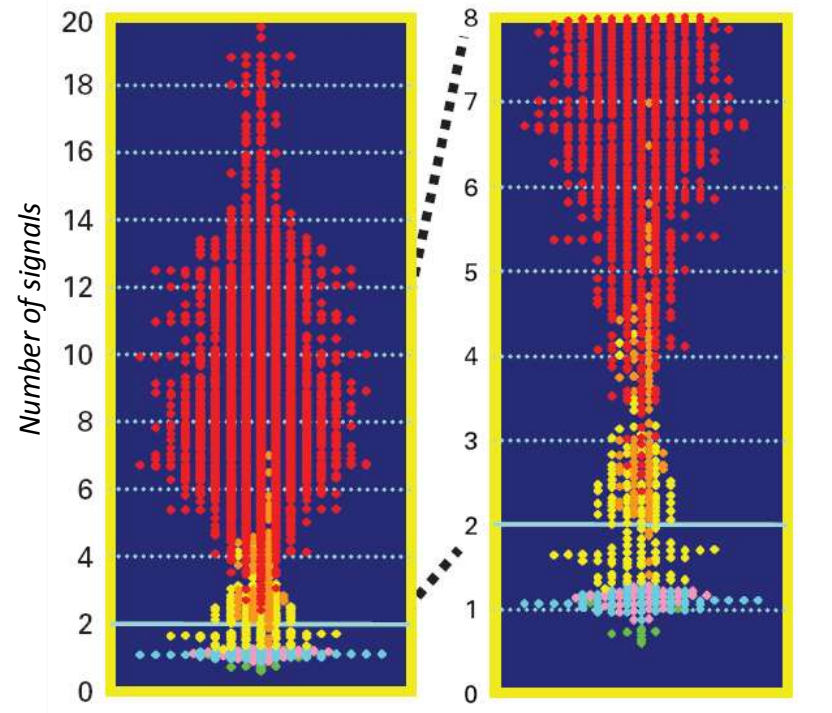
Amplified *HER2*, +17: Amplified *HER2* and +CEP17.

Amplified *HER2*, -17: Amplified *HER2* and -CEP17.

+17:  $\geq 3$  *HER2* and CEP17 copies in > 30% of invasive component (ratio > 0.80 and < 1.30).

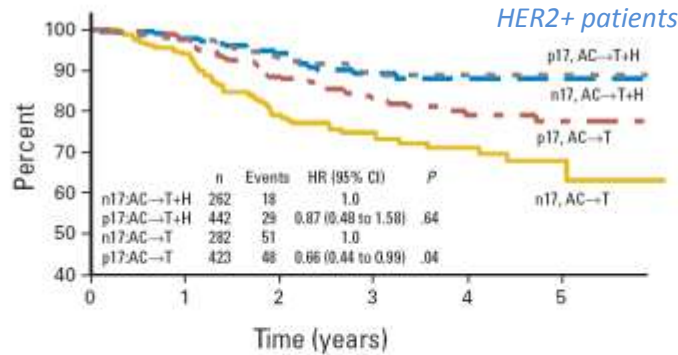
-17: 1 *HER2* and CEP17 copy in > 60% of invasive component (ratio > 0.80 and < 1.30).

Abbreviations: CEP17, centromere enumerator probe for chromosome 17; NACA, no apparent chromosome abnormality.

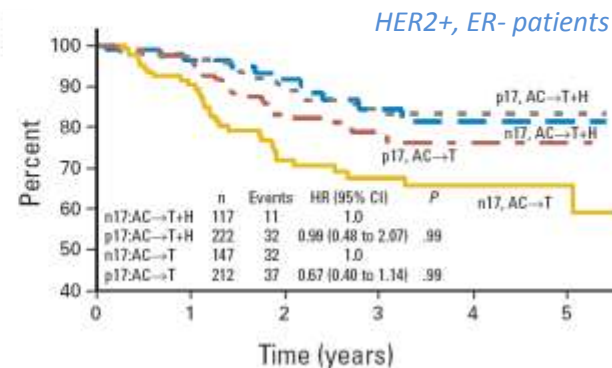
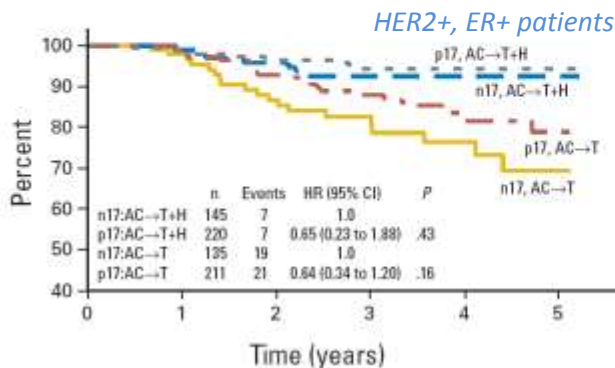
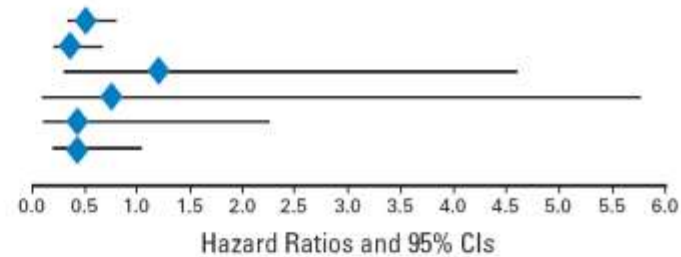


Anomaly	n	%
Amplified <i>HER2</i>	1,488	78.8
p17	865	45.8
m17	544	28.8
m17	79	4.2
Small Clone	85	4.5
Dup <i>HER2</i>	118	6.3
p17/non-amp	37	2.0
m17/- <i>HER2</i>	10	0.5
NACA	57	3.0
Failed/ND	93	4.9
Total	1,888	100

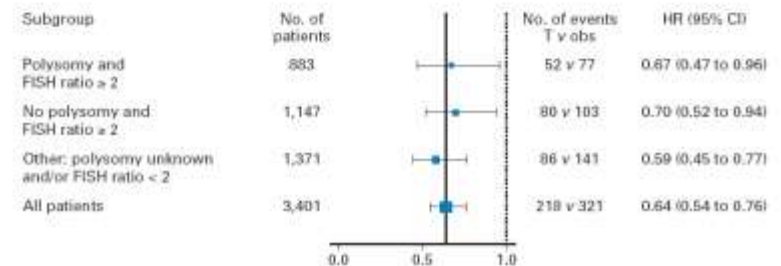
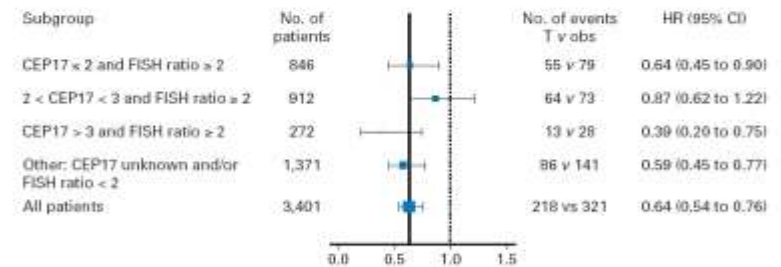
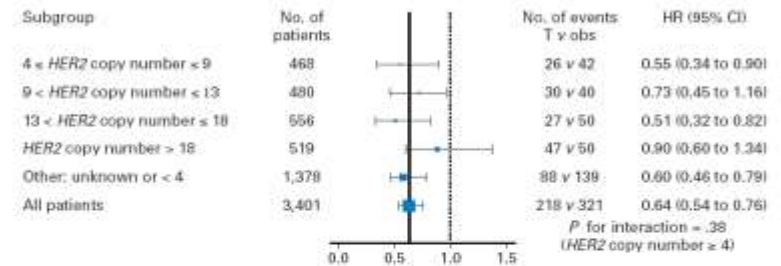
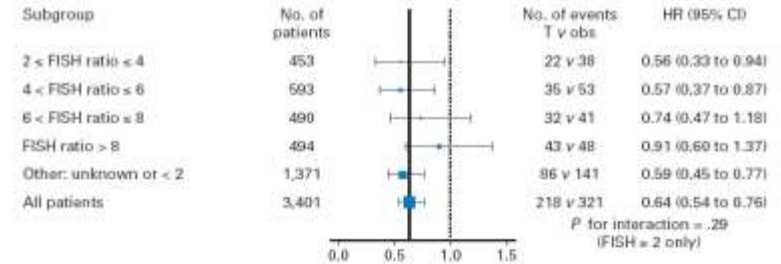
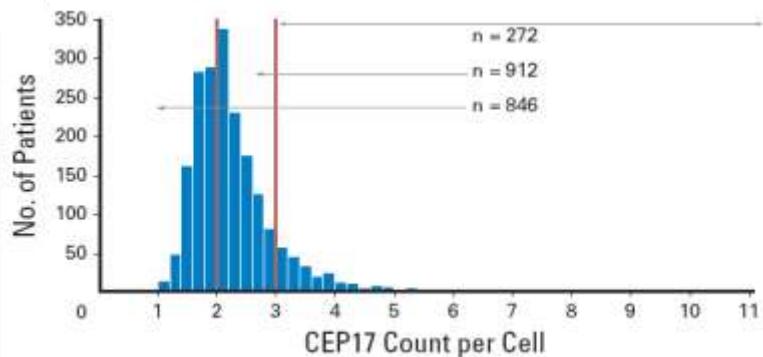
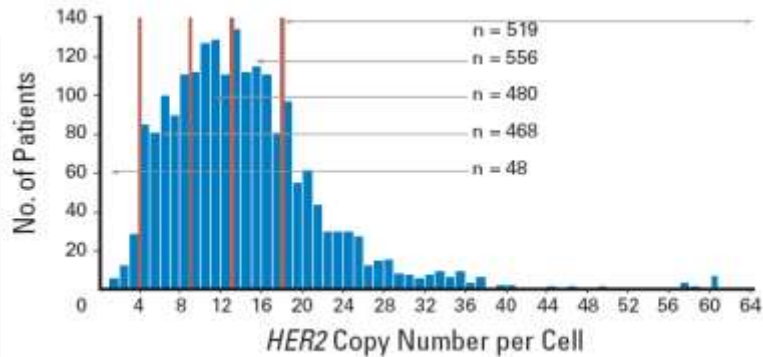
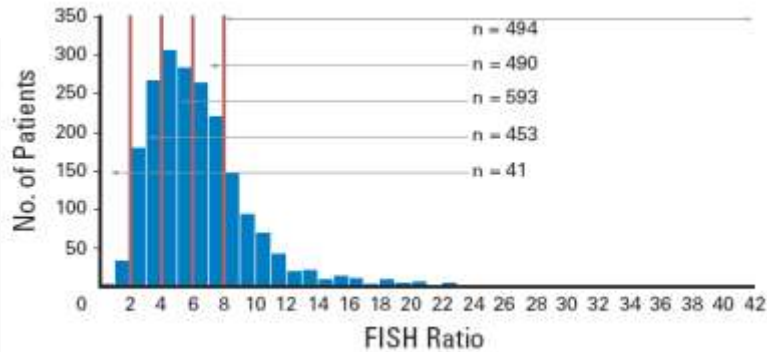
# HER2 gene copy number does not predict response to trastuzumab: the N9831 adjuvant trial (n=1888)



Subgroup	n	HR*	95% CI	P
<b>IHC or FISH HER2/CEP17 RATIO</b>				
Amp w/ p17	865	0.52	0.33 to 0.82	.0006
Amp w/ n17	544	0.37	0.21 to 0.64	.0004
Amp w/ m17	79	1.21	0.32 to 4.62	.78
Amp w/ sc	85	0.80	0.10 to 5.79	.80
Duplicated	118	0.33	0.09 to 2.26	.33
Not Amp	104	0.44	0.19 to 1.05	.07



# HER2 gene copy number does not predict response to trastuzumab: the HERA trial (n=2071)





# Chromosome 17 polysomy without *HER2* amplification not predict response to trastuzumab or lapatinib

## Key Inclusion

- Incurable Stage III/IV
- No prior treatment for MBC
- **HER-2 negative or untested**

N = 579

R  
A  
N  
D  
O  
M  
I  
Z  
A  
T  
I  
O  
N

Paclitaxel 175 mg/m<sup>2</sup> q3w  
Lapatinib 1,500 mg po QD  
(N = 291)

Paclitaxel 175 mg/m<sup>2</sup> q3w  
Placebo po QD  
(N = 288)

## Stratification

- Disease sites
- Stage of disease

## Endpoints

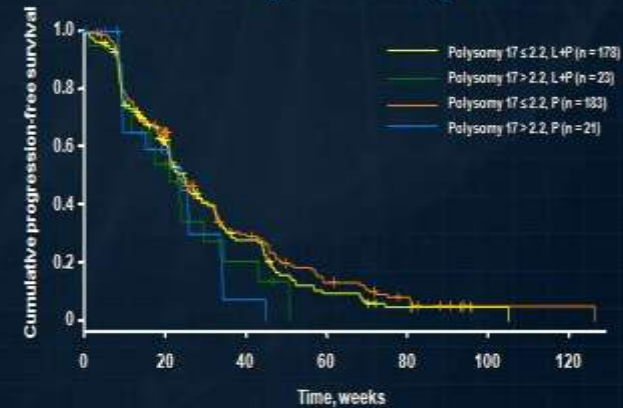
- Primary: TTP
- Secondary: PFS, OS, QoL
- Safety

## Comparison of Progression-Free Survival Among HER2-Negative Patients Based on Polysomy

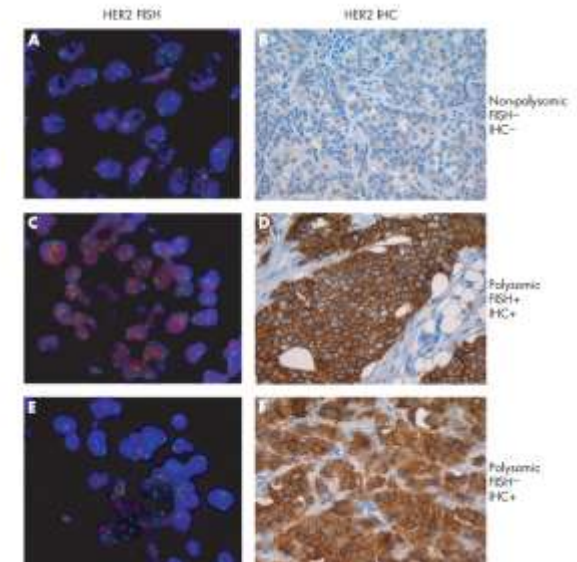
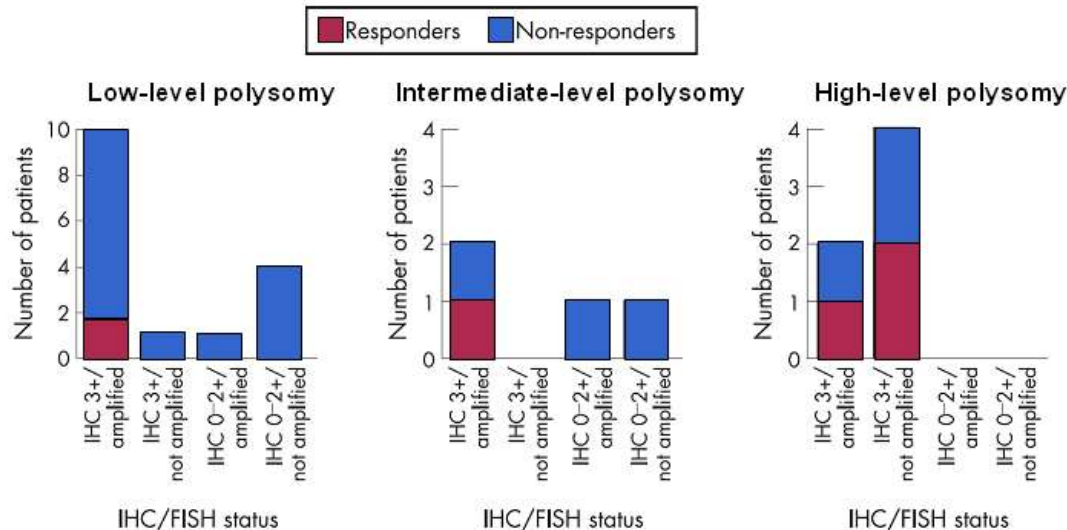
- HER2-negative (FISH), n = 405
  - Polysomy\*, n (%) = 44 (11)
  - No polysomy, n (%) = 361 (89)

	Lapatinib + Paclitaxel		Paclitaxel	
	Polysomy	No polysomy	Polysomy	No polysomy
Median PFS, weeks	20.9	24.6	24.4	23.1
95% CI	11.4-29.3		9.3-33.6	

\*Chr 17 ≥ 2.2



Downey, L et al. *Clin Cancer Res*, 2010



Hofmann, M et al. *J Clin Pathol*, 2008



# Poor prognostic significance of unamplified chromosome 17 polysomy in breast cancer

<i>Prognostic and predictive factors</i>	<i>N (%)</i>	<i>P (%)</i>	<i>A (%)</i>	<i>N vs P</i>	<i>P vs A</i>
Nottingham score-8	11	37	34	<i>P</i> = 0.008	—
Nottingham score-9	11	14	34	—	<i>P</i> = 0.033
Nuclear grade-3	26	61	74	<i>P</i> = 0.002	—
Mitotic score-2	18	54	43	<i>P</i> = 0.001	—
Mitotic score-3	18	26	40	—	—
Histologic grade 3	20	44	69	<i>P</i> = 0.02	<i>P</i> = 0.02
T stage-2	18	31	30	—	—
Lymph vascular invasion present	17	19	34	—	—
Positive LN	38	42	45	—	—
ER negativity	11	30	50	<i>P</i> = 0.04	<i>P</i> = 0.05
PR negativity	24	35	34	—	—

— = not significant.

## PATOLOGÍA MAMARIA

CO-3 (ANFITEATRO)

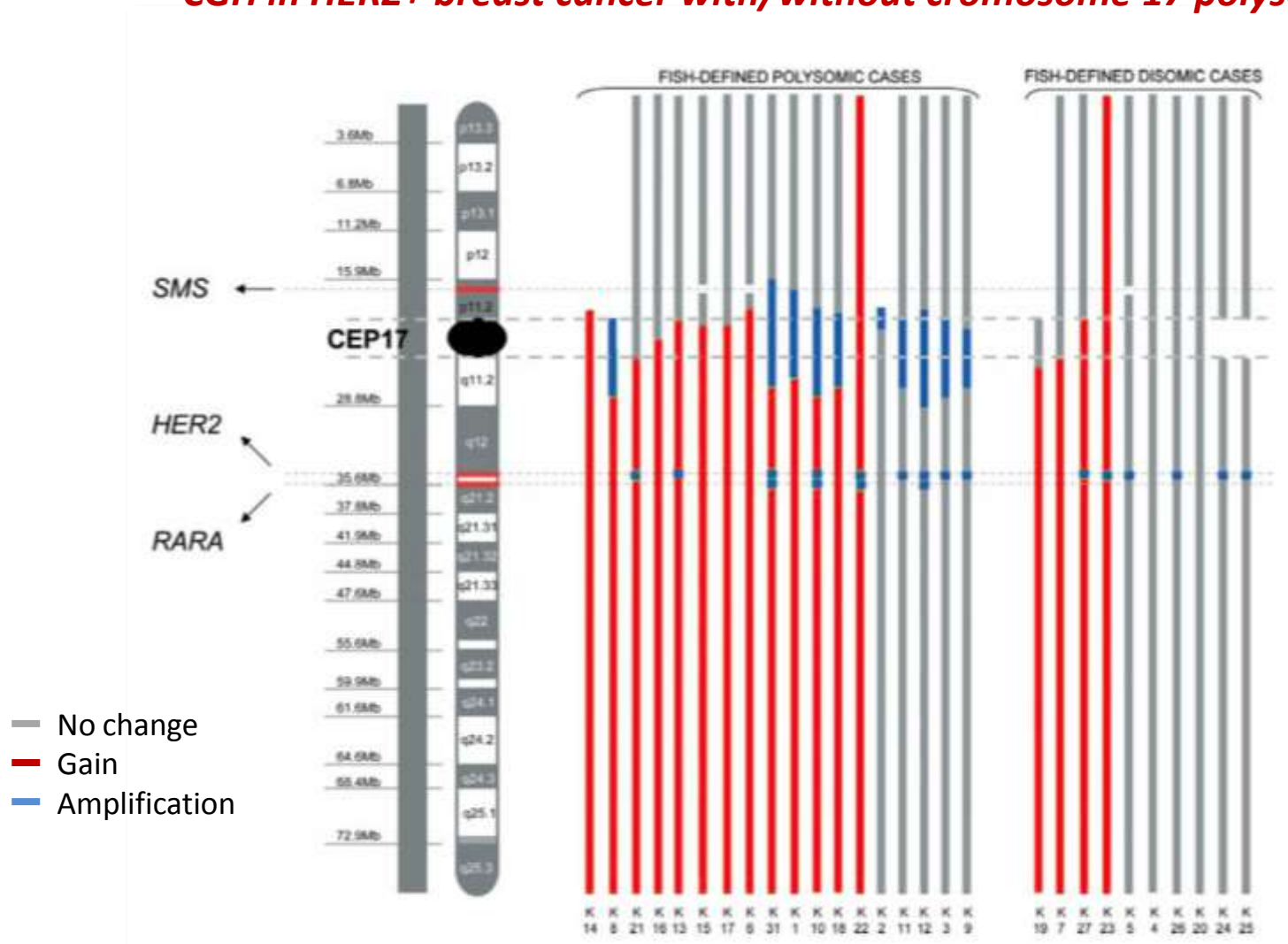
**LA POLISOMÍA 17 SE ASOCIA A FACTORES PRONÓSTICOS ADVERSOS EN EL CARCINOMA DUCTAL INFILTRANTE DE MAMA**

A GAAFAR, L ANDRÉS, J GONZÁLEZ DE TÁNAGO, FJ ORTEGA, JJ BURGOS, JI LÓPEZ

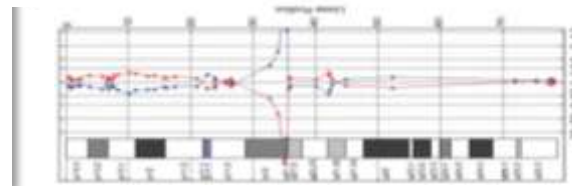
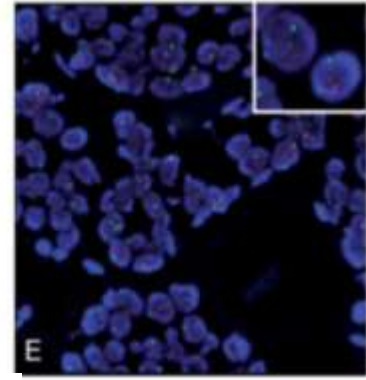
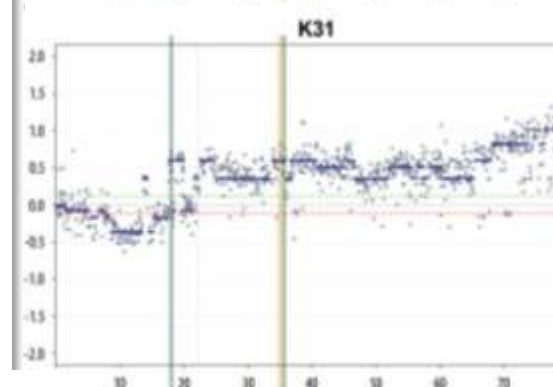
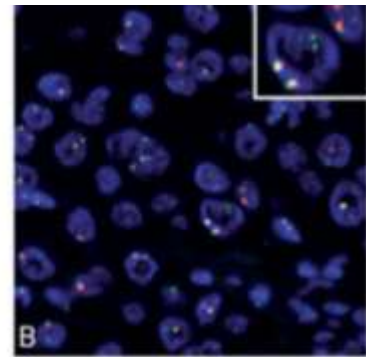
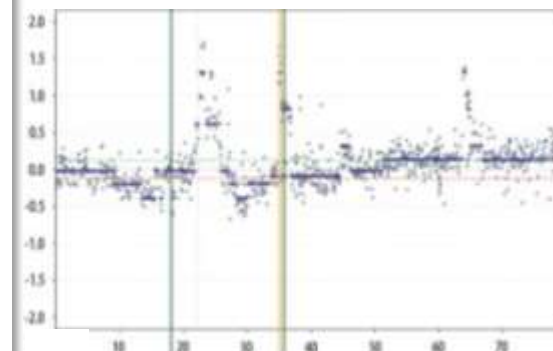
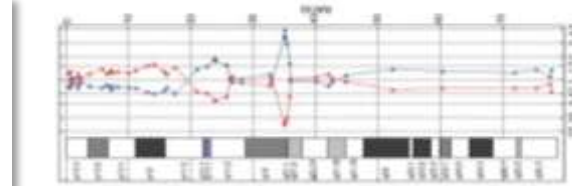
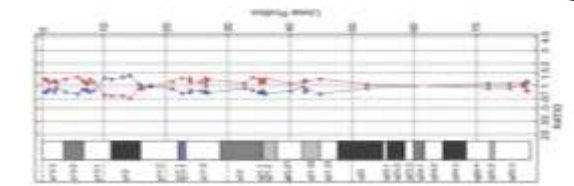
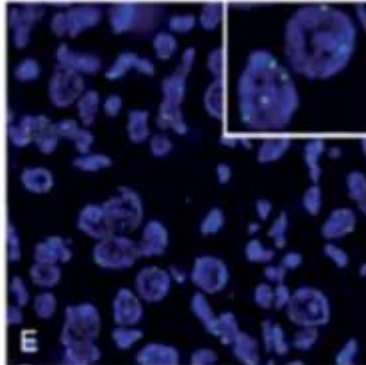
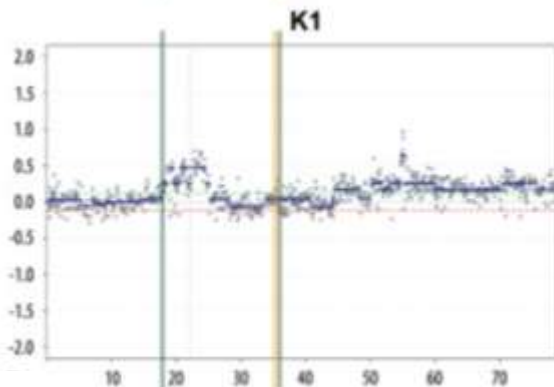
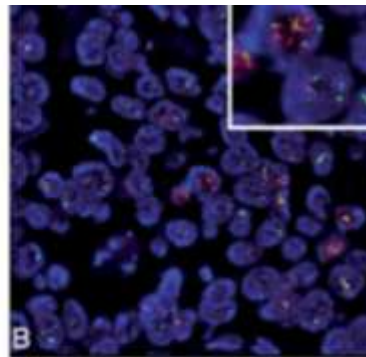
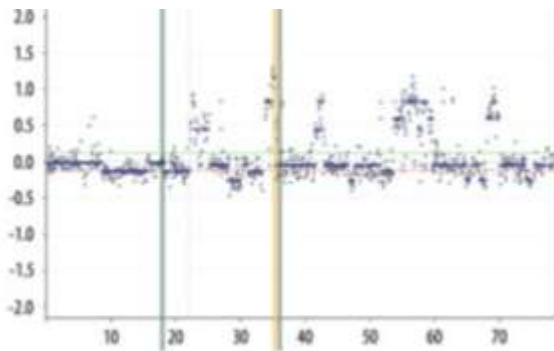
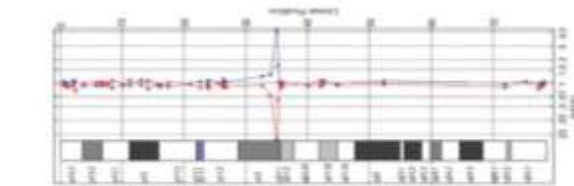
*Servicio de Anatomía Patológica, Hospital Universitario de Cruces (UPV/EHU), Barakaldo, Bizkaia*

# Does chromosome 17 centromere copy number predict polysomy in breast cancer?

## CGH in HER2+ breast cancer with/without chromosome 17 polysomy



# Does chromosome 17 centromere copy number predict polysomy in breast cancer?





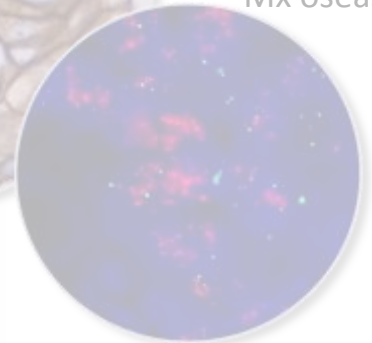
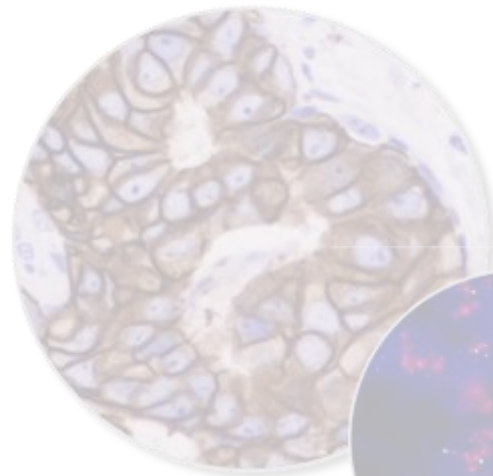
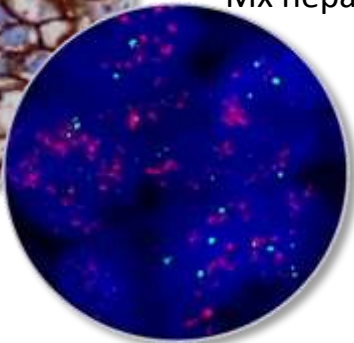
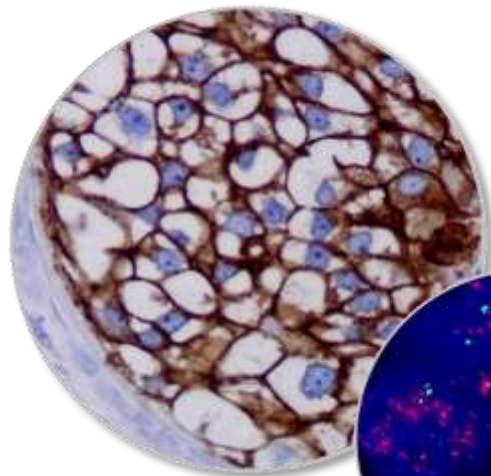
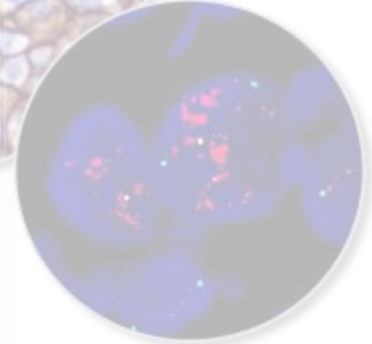
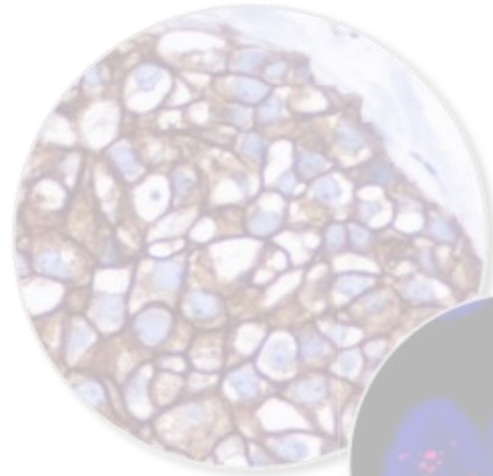
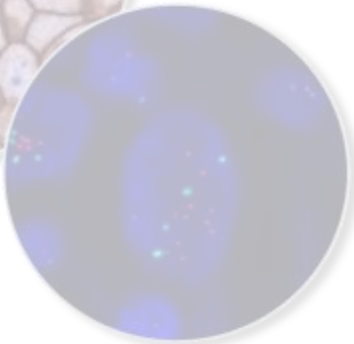
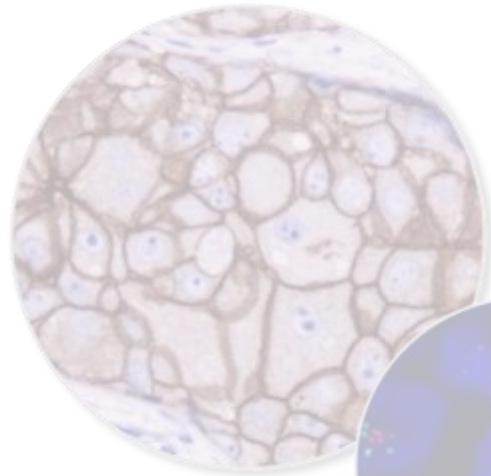
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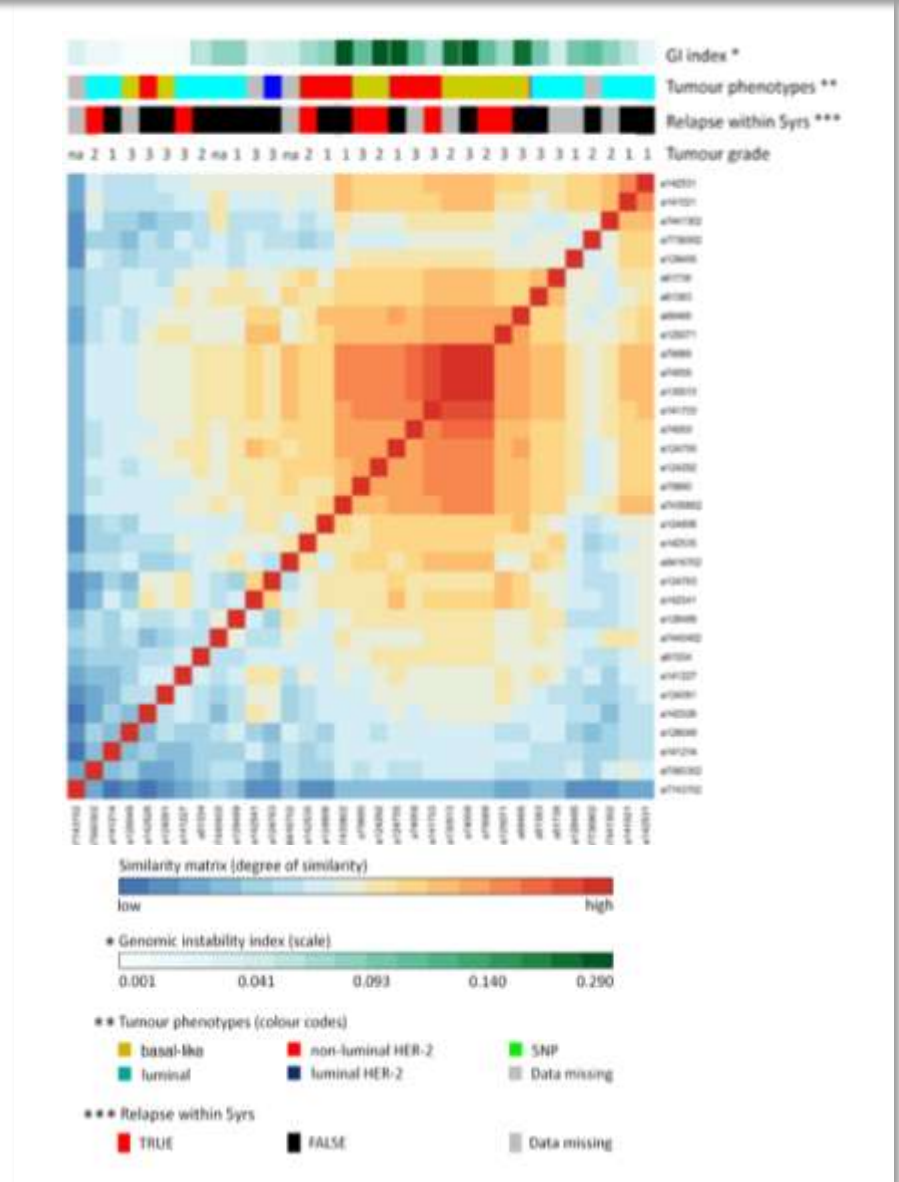
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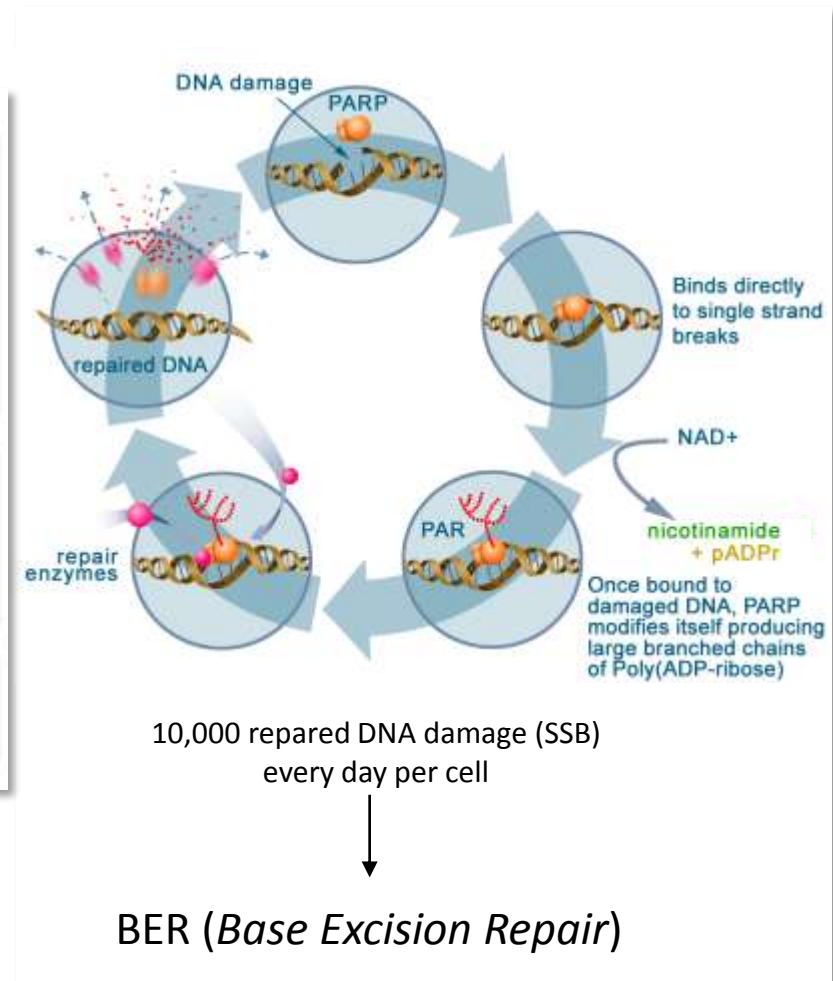
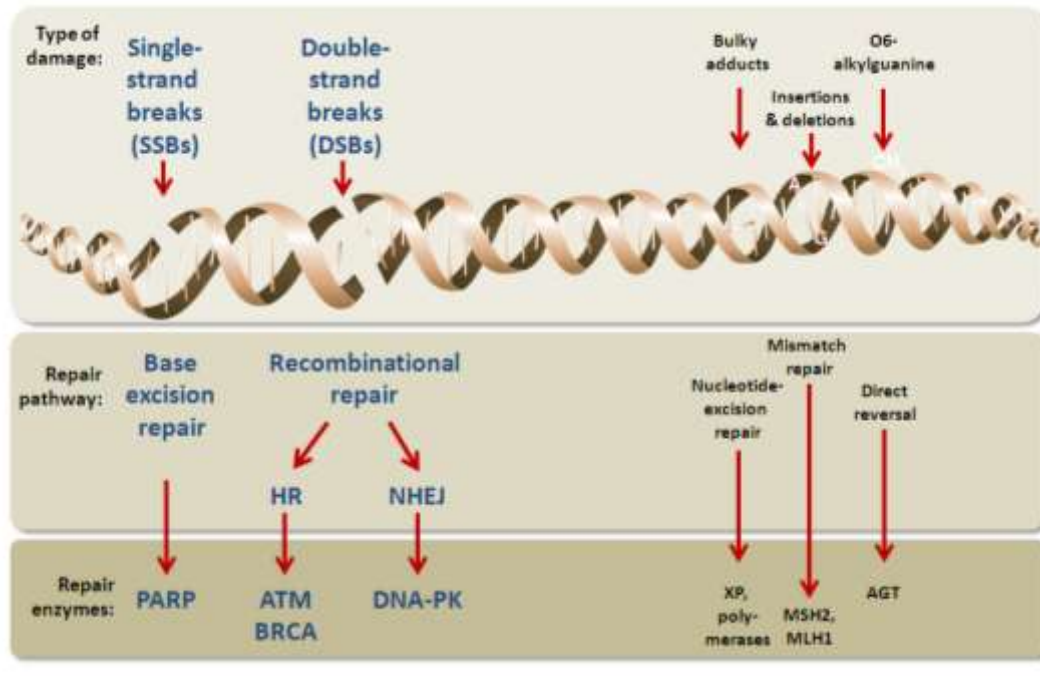


# Amplification of HER2 is a marker for global genomic instability



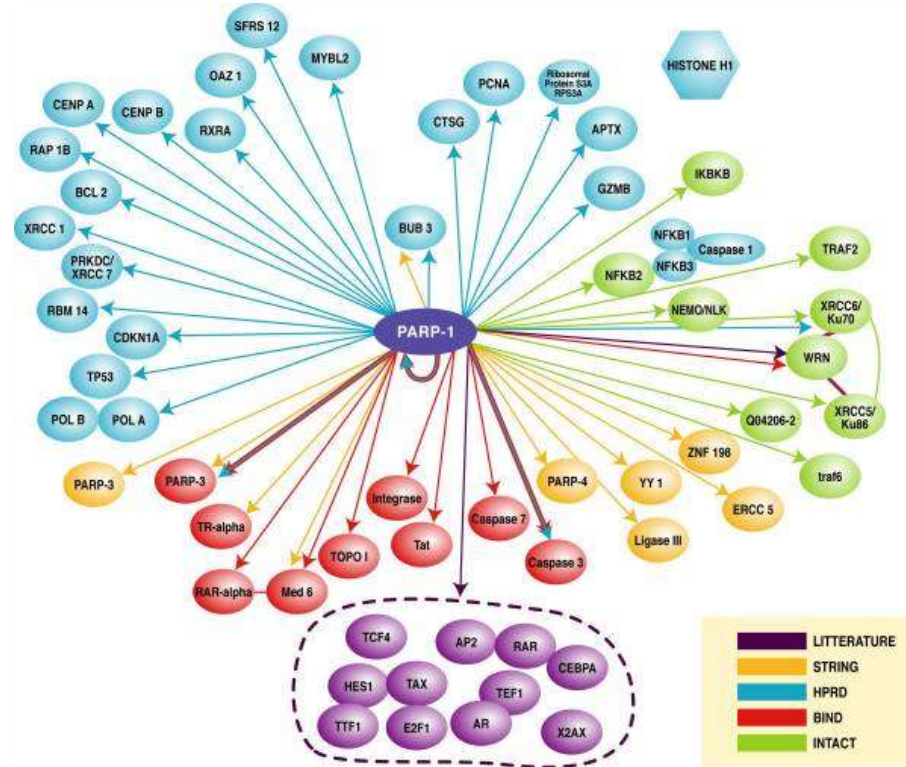
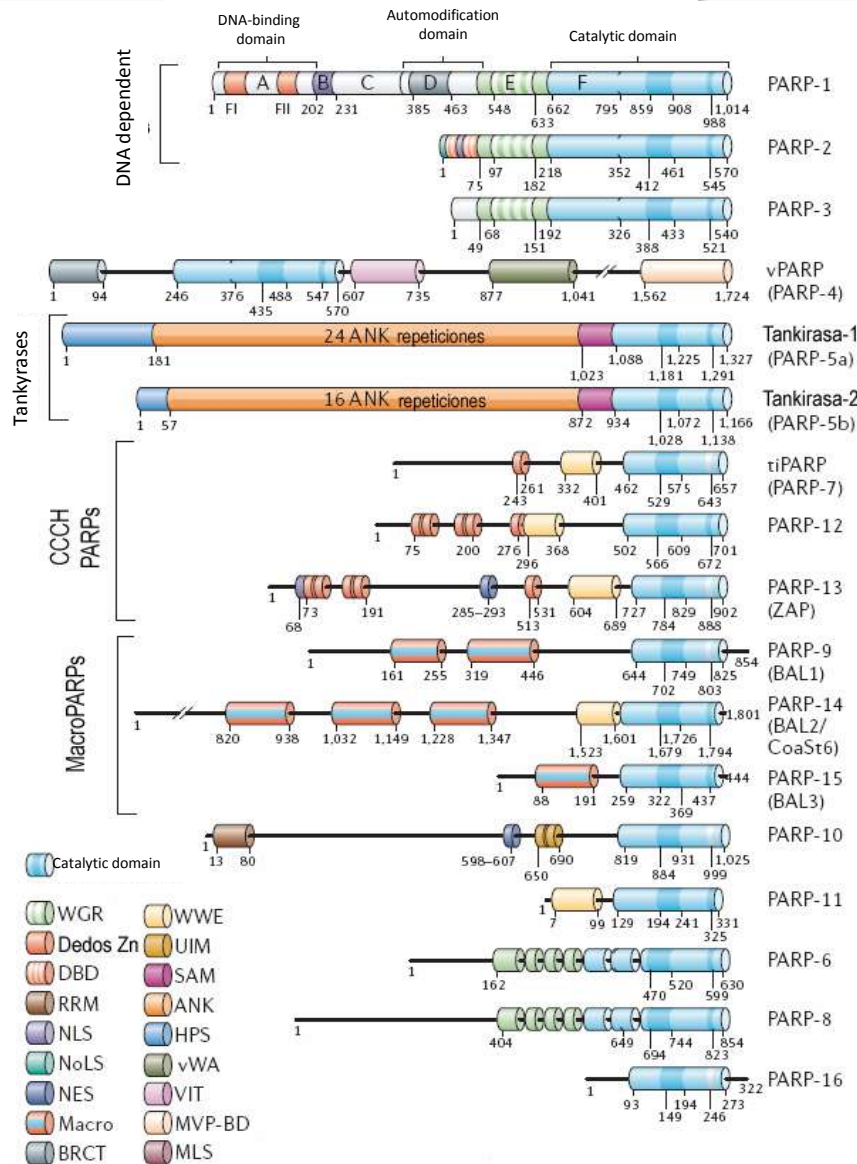
# Poly (ADP-Ribose) Polymerase repairs single strand DNA breaks

## Types of DNA damage and repair





# Poly(ADPribose) Polymerases Family



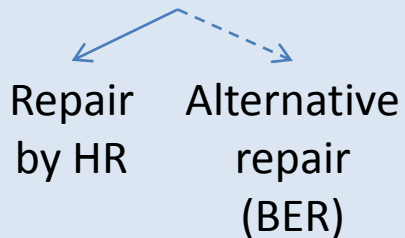
# The synthetic lethality concept

<b>Gene X</b>	<b>Gene Y</b>	
+	+	<b>No effect</b>
-	+	<b>No effect</b>
+	-	<b>No effect</b>
-	-	<b>Death</b>

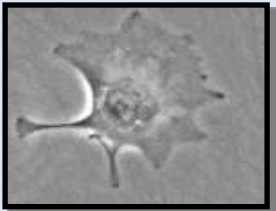
# The synthetic lethality concept



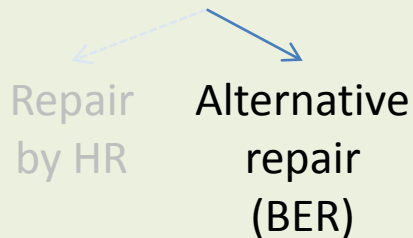
## Normal Cells



Genomic stability  
Survival



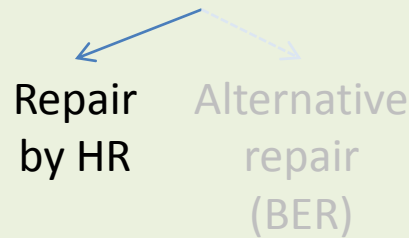
## BRCA-Deficient Cells



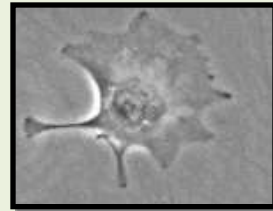
Gross genomic instability  
Survival



## PARP-Deficient Cells



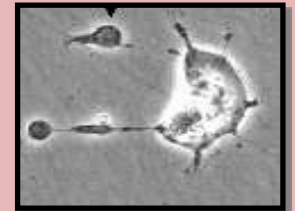
Gross genomic instability  
Survival



## BRCA and PARP Deficient Cells

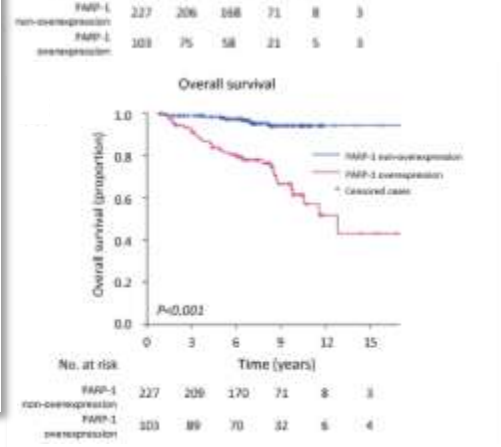
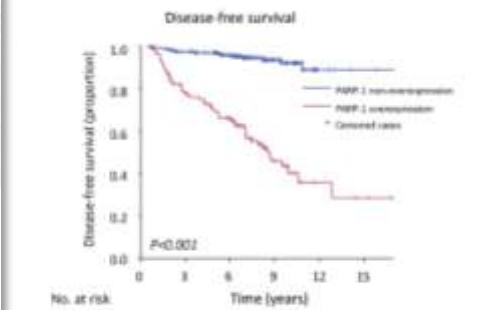
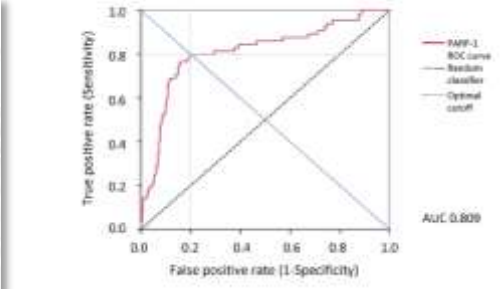
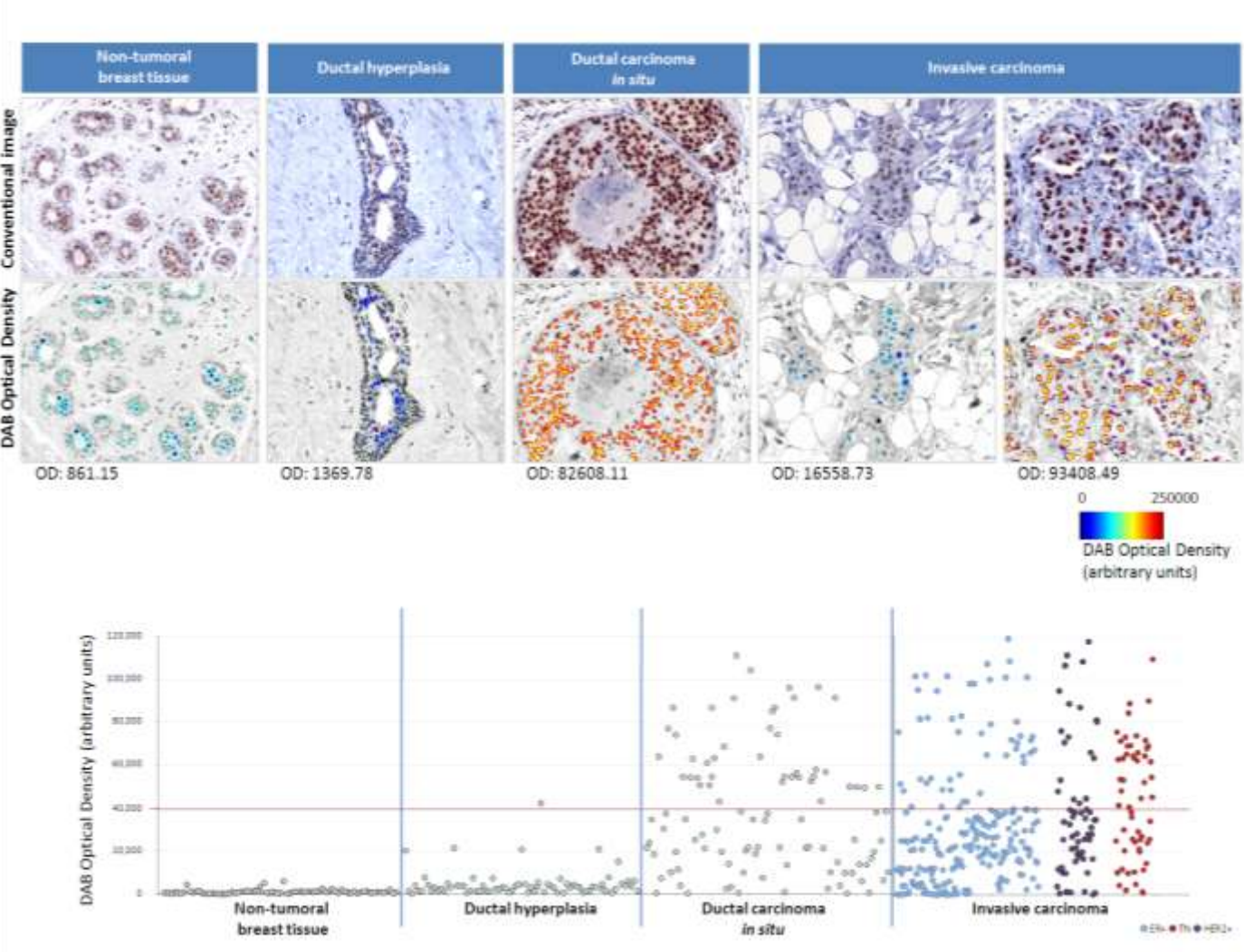


Cell death with chromosomal deletions or exchanges

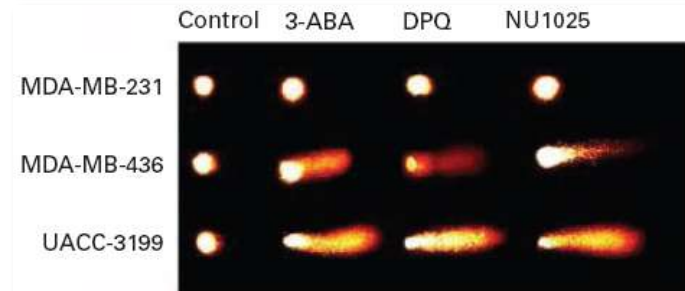
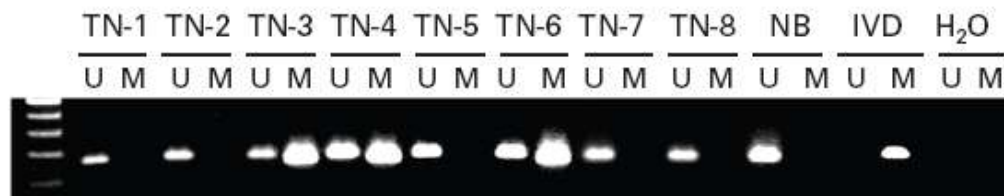
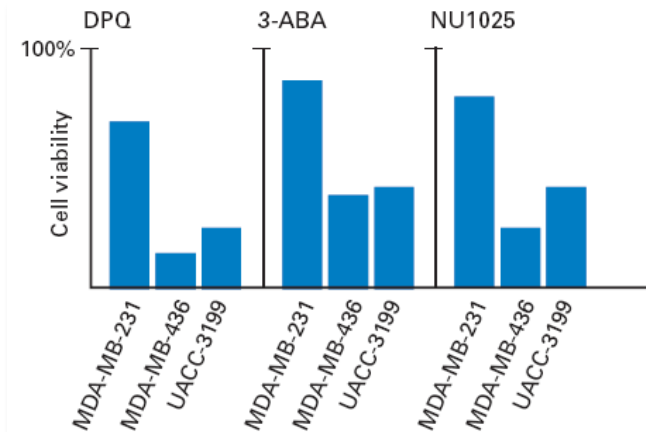
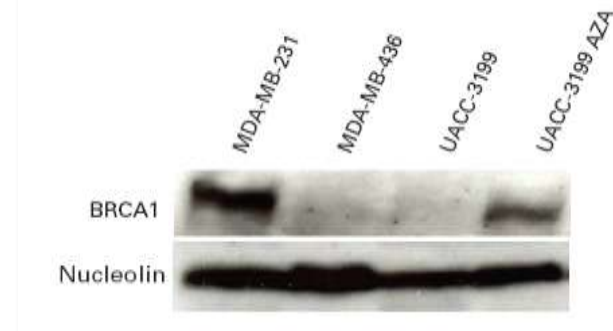
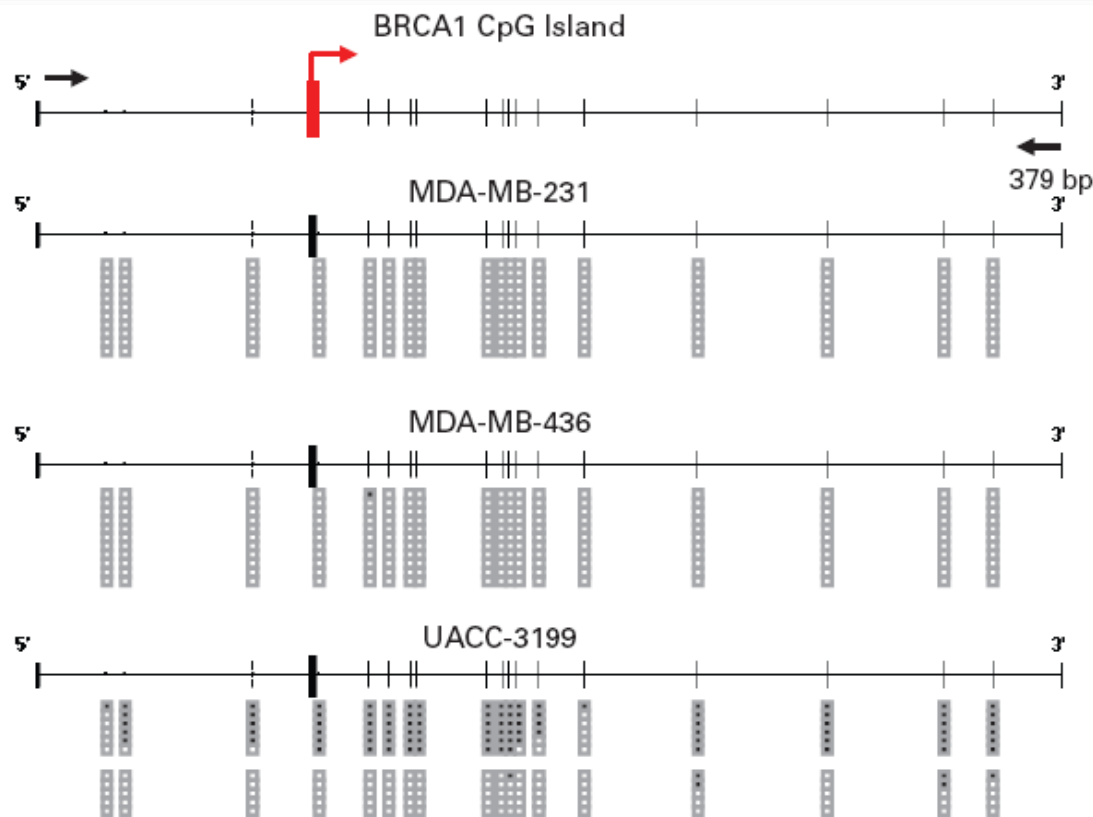




# PARP1 overexpression in breast cancer predicts survival and correlates with genomic instability

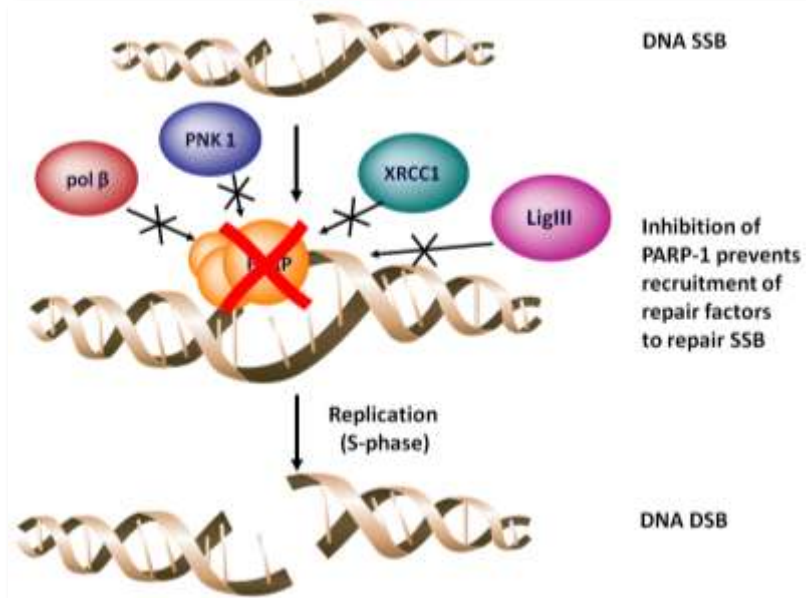


# BRCA1 CpG island hypermethylation and silencing occurs in breast cancer



# Inhibiting PARP-1 increases double-strand DNA damage

## PARP1 inhibitors in clinical development

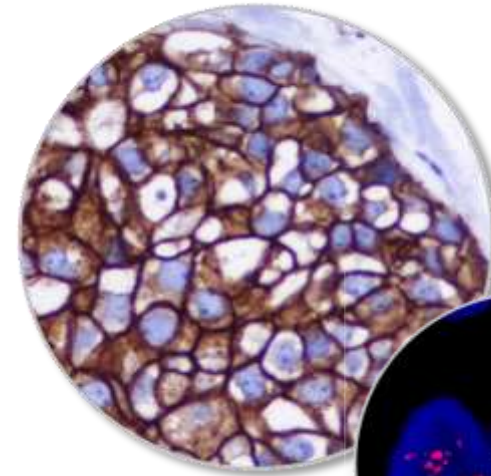


Drug	Company	Biophysical parameters	Synergizes with (in vitro)	Clinical trials*	Phase*
ABT-888	Abbott	$K_i = 5.2$ nM (PARP1) $K_i = 2.9$ nM (PARP2) $EC_{50} = 2$ nM (C41 cells)	Temozolomide Platins Cyclophosphamide Ionizing radiation MNNG Topoisomerase I poisons	Glioblastoma multiforme (with temozolomide) Solid tumours and leukaemia (various combinations) BRCA1- or BRCA2- mutant tumours	Phase II Phase I Phase I
AG014699	Pfizer	$K_i = 1.4$ nM (PARP1)	Temozolomide Ionizing radiation Topotecan	BRCA1- or BRCA2- mutant tumours	Phase II
AZD2281 (olaparib)	AstraZeneca	$IC_{50} = 5$ nM (PARP1) $IC_{50} = 1$ nM (PARP2) $IC_{50} = 1.5$ $\mu$ M (tankyrase 1)	Temozolomide Platins MMS Ionizing radiation (with and without 17-AAG)	Platin-sensitive ovarian cancer BRCA1- or BRCA2- mutant tumours (with carboplatin) Triple-negative breast cancer (single-agent or with carboplatin) Other solid tumours	Phase II Phase II Phase II Phase I/II
BSI-201	Sanofi-Aventis	ND	Ionizing radiation Oxaliplatin Gemcitabine and carboplatin Topotecan	Triple-negative breast cancer (with gemcitabine and carboplatin) Ovarian cancer, glioblastoma multiforme and uterine cancer (various combinations) BRCA2- mutant pancreatic cancer (various combinations) Other solid tumours	Phase III Phase II Phase Ib Phase I/II
CEP-8983/ CEP-9722 (prodrug)	Cephalon	$IC_{50} = 20$ nM (PARP1) $IC_{50} = 6$ nM (PARP2)	Temozolomide Topoisomerase I poisons	Solid tumours (with temozolomide)	Phase I
MK-4827	Merck	$IC_{50} = 3.2$ nM (PARP1) $IC_{50} = 4$ nM (PARP2)		Solid tumours and ovarian cancer	Phase I

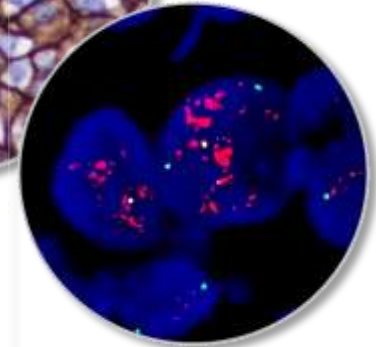


# Cuatro carcinomas de mama con sobreexpresión de HER2

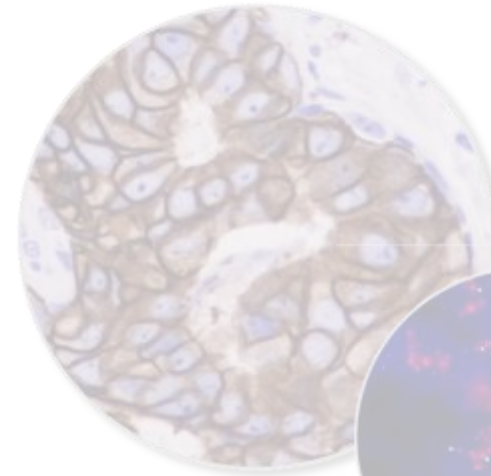
54 años  
CDI 20mm, pN0  
ER+/PR-  
HER2 3+, ratio 3  
FACx6  
Herceptin 1 año  
No recidiva tras 8 años



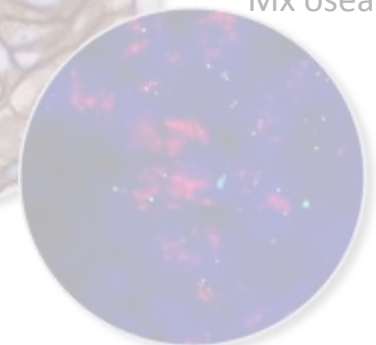
47 años  
CDI 18mm, pN0  
ER+/PR+  
HER2 3+, ratio >15  
FACx6  
Herceptin 1 año  
Recidiva local a 4 años



52 años  
CDI 22mm, pN0  
ER-/PR-  
HER2 3+, ratio 7  
FACx6  
Herceptin 1 año  
Mx hepáticas a 3 años

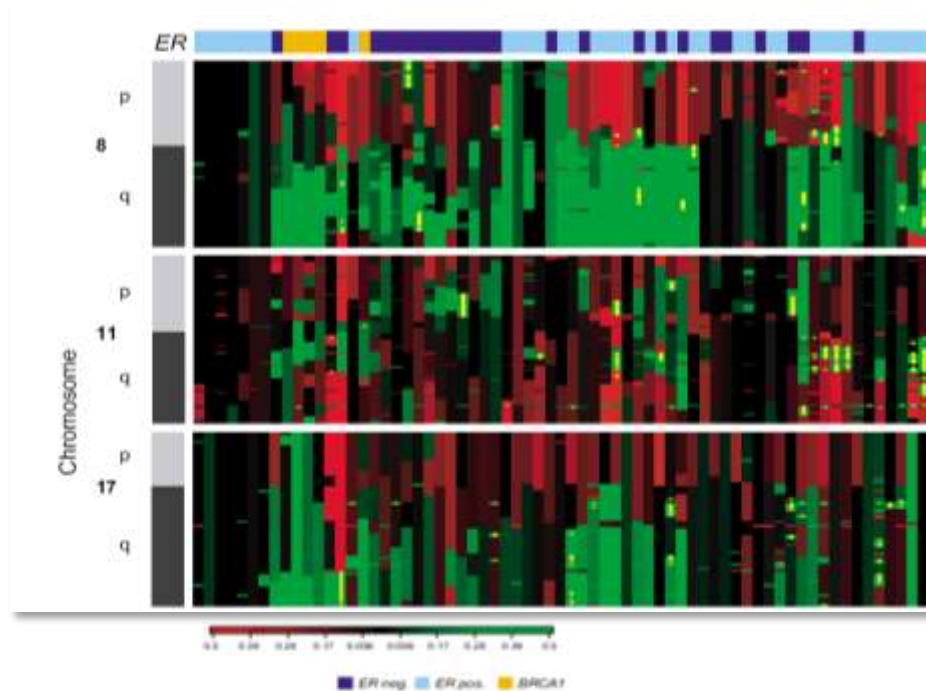
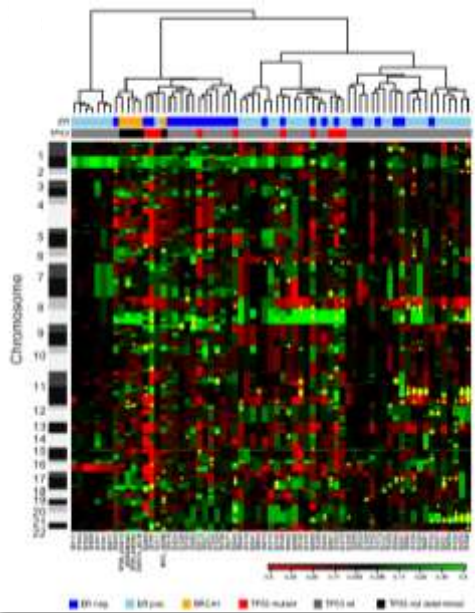


49 años  
CDI 18mm, pN0  
ER+/PR-  
HER2 3+, ratio >15  
FACx6  
Herceptin 1 año  
Mx óseas a 4 años





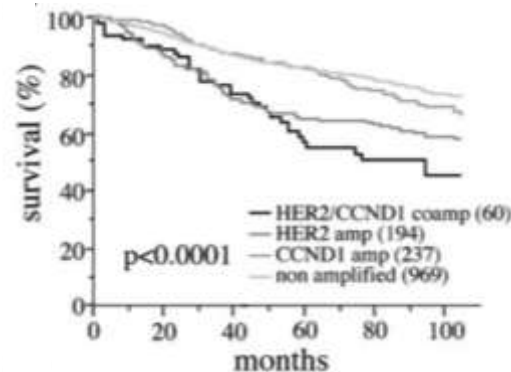
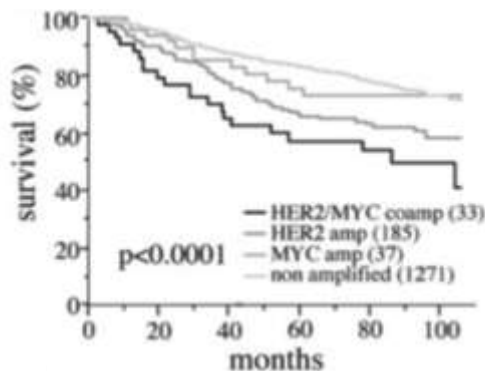
# High resolution genomic of copy number aberrations in HER2-amplified breast cancer



FGFR  
Ikb  
PRDM14  
MTDH  
**MYC**

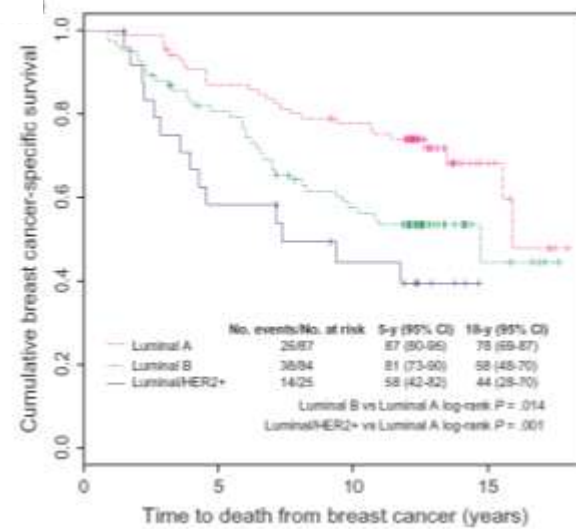
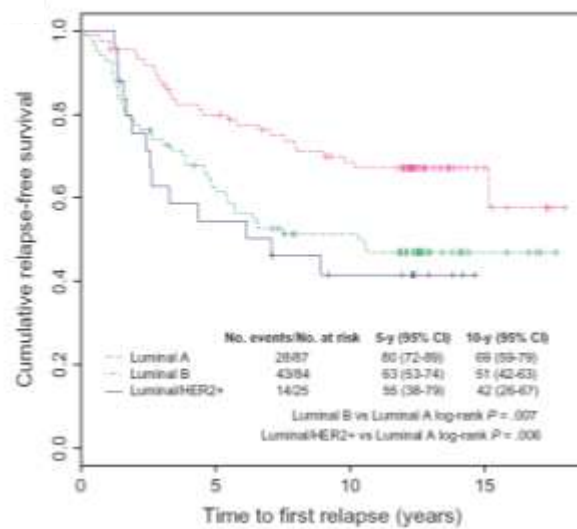
EMSY  
**CCND1**

TP53  
TRAF4  
CPD  
MED1  
**HER2**  
GRB7  
CDC6  
**TOP2A**  
MAPT  
BIRC5  
STAT3  
BRCA1

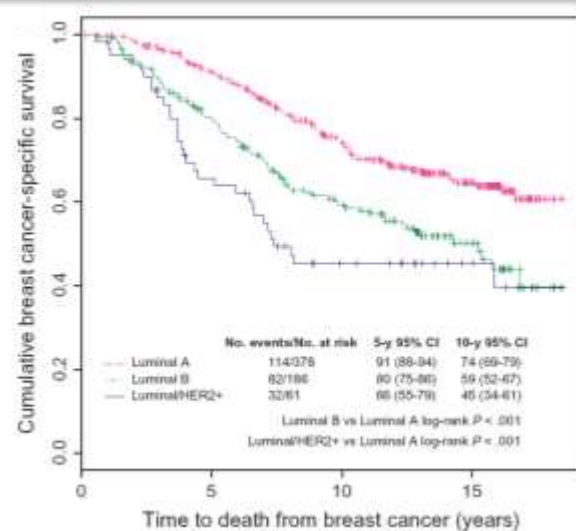
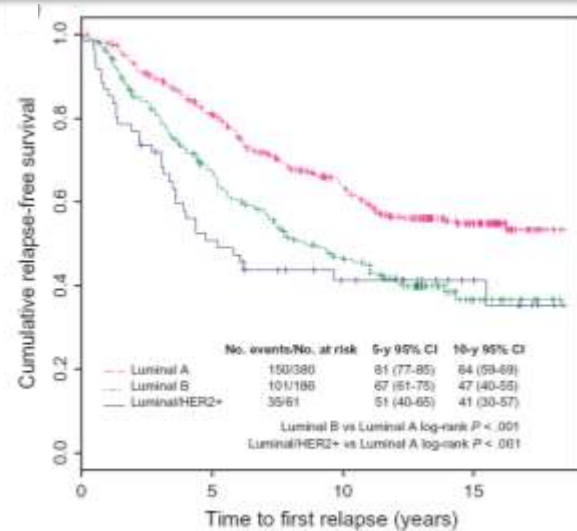


# Prognosis of HER2 patients with hormone receptor expression

*N=196, CT-treated*

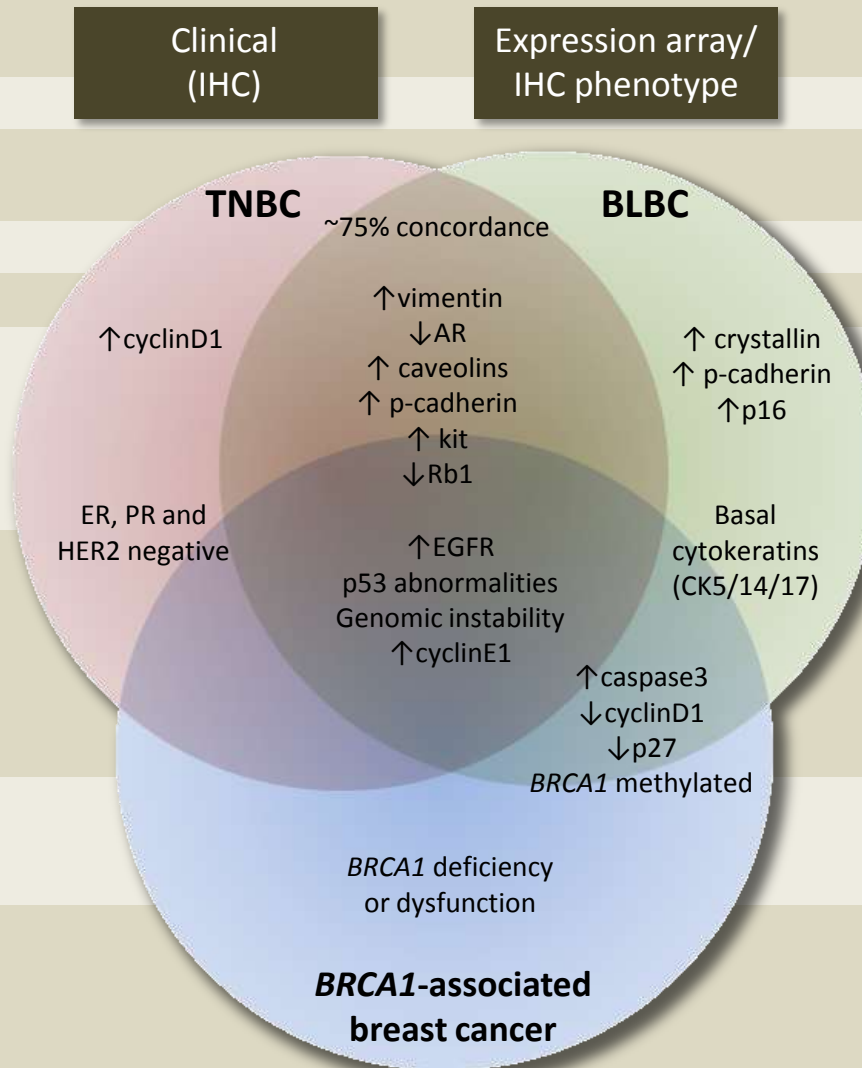


*N=976, HT-treated*



# HER2 in basal-like breast cancer

- 10-17% of tumors (depending threshold of IHC)
- 100% TN
- 8-29% are normal-like, apocrine or claudin-low
- <50 years
- Often present as interval cancer
- Aggressive behavior: peak of risk of recurrence 1-3 years after dx and majority of deaths in first 5 years following therapy
- No correlation between tumor size and presence of lymph node metastasis
- 10% grade I, apocrine, pleomorphic lobular, mixed carcinomas
- Elevated mitotic count, high apoptotic raise, central fibrosis, 78% pushing margin, 46% lymphocytic response, metaplasia
- 78% genomic instability



- 15% of tumors
- 80-100% TN
- 15-45% express ER, 14% express HER2
- Young patients
- Often present as interval cancer
- More aggressive clinical behavior when compared with either ER, non-basal-like cancers or with grade-matched non-basal-like cancers
- Disseminate to axillary nodes and bones less frequently and to favor a hematogenous spread with a peculiar proclivity to develop metastatic deposits in the brain and lungs
- High grade ductal, medullary, adenoid-cystic, secretory, metaplastic carcinoma
- 76% elevated mitotic count, high apoptotic raise, 51% central necrosis, 34% pushing margin, 61% lymphocytic response, metaplasia
- 82% genomic instability

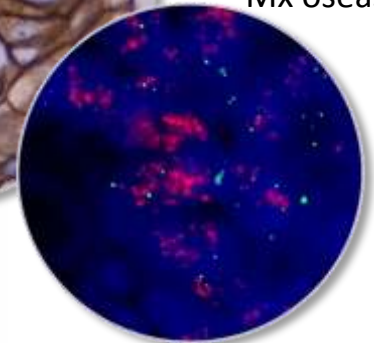
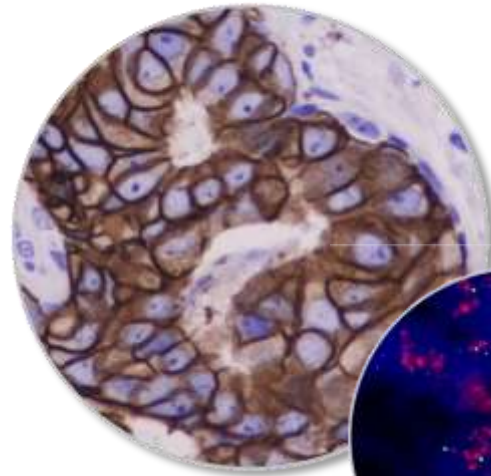
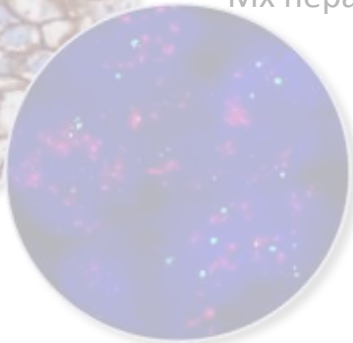
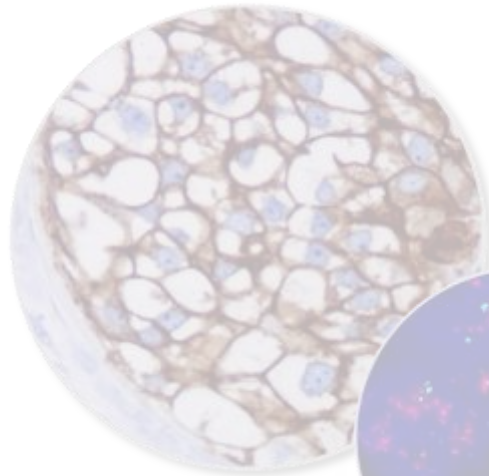
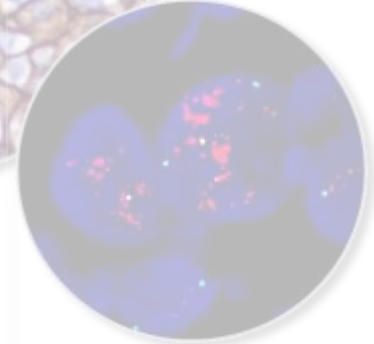
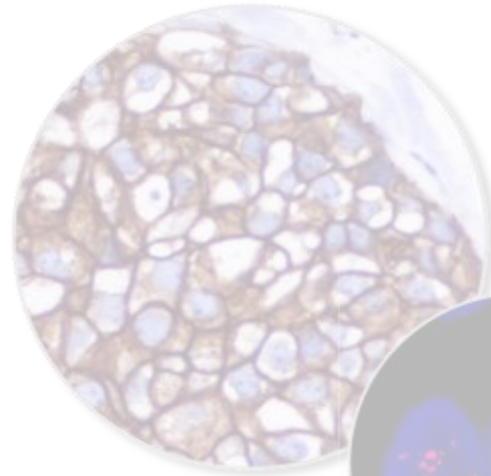
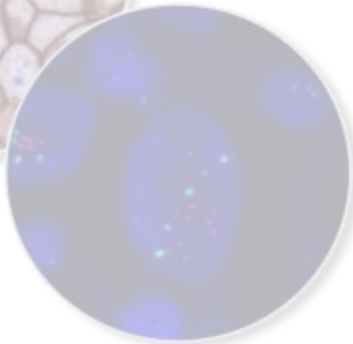
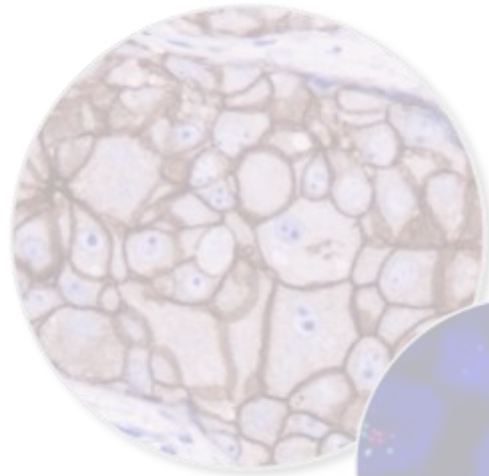
# Cuatro carcinomas de mama con sobreexpresión de HER2

54 años  
CDI 20mm, pN0  
ER+/PR-  
HER2 3+, ratio 3  
FACx6  
Herceptin 1 año  
No recidiva tras 8 años

47 años  
CDI 18mm, pN0  
ER+/PR+  
HER2 3+, ratio >15  
FACx6  
Herceptin 1 año  
Recidiva local a 4 años

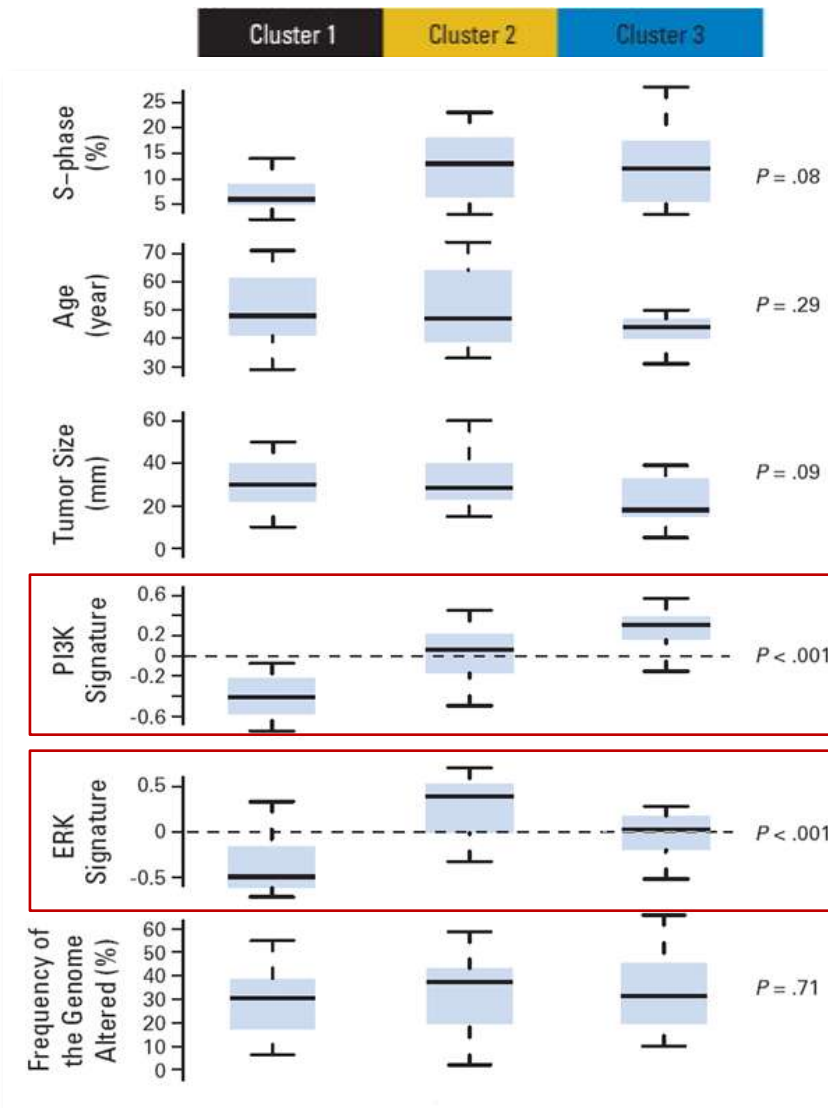
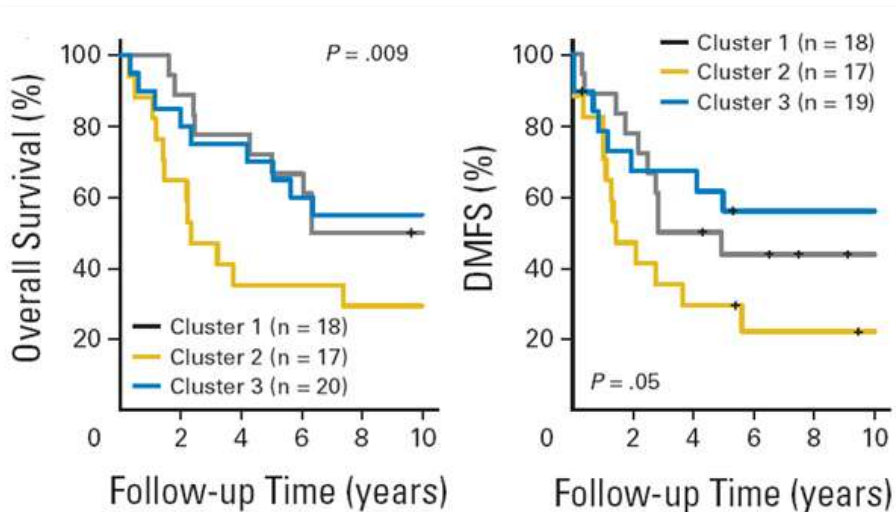
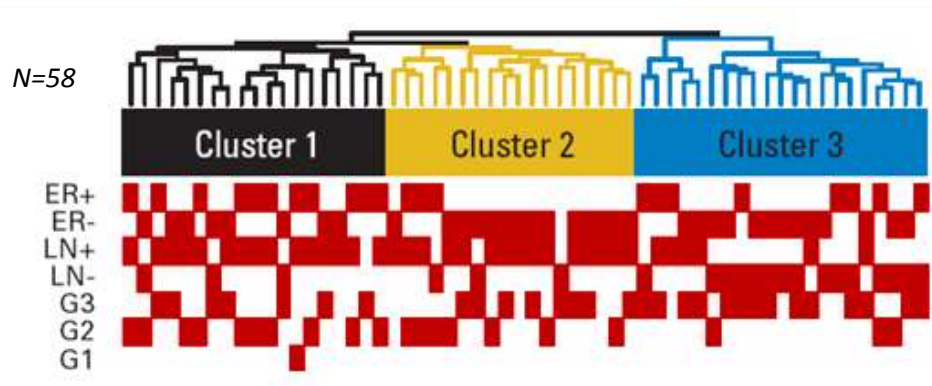
52 años  
CDI 22mm, pN0  
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Herceptin 1 año  
Mx hepáticas a 3 años

49 años  
CDI 18mm, pN0  
ER+/-PR-  
HER2 3+, ratio >15  
FACx6  
Herceptin 1 año  
Mx óseas a 4 años

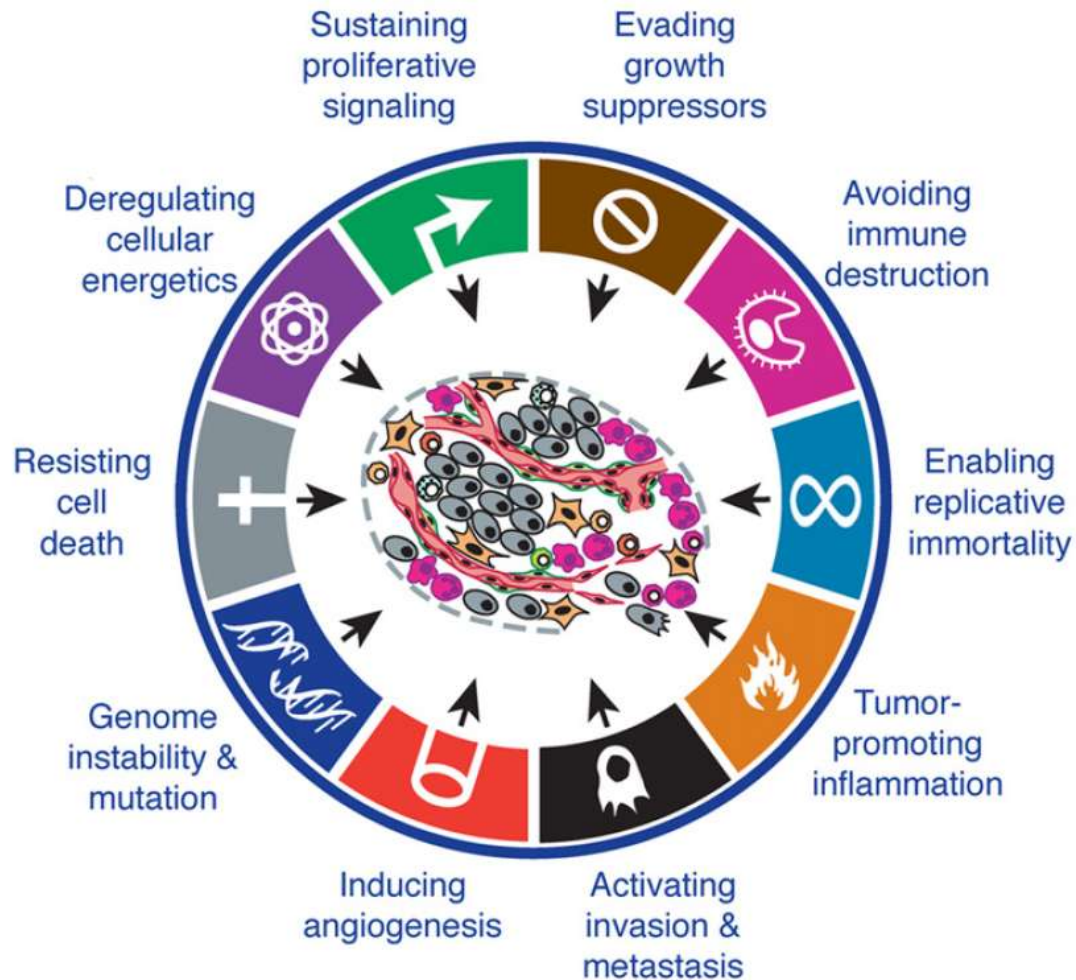




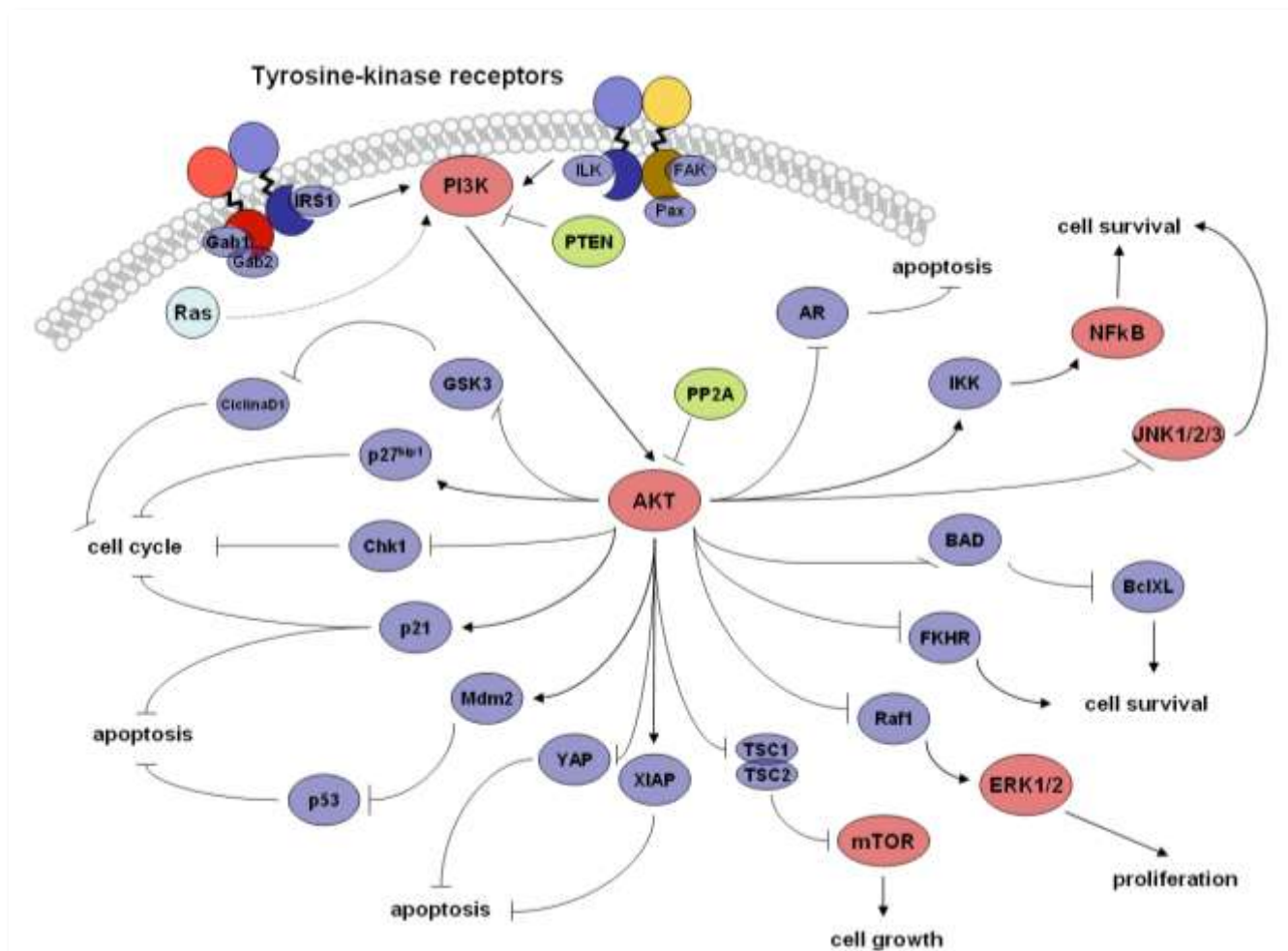
# Identification of subtypes in HER2 amplified breast cancer reveals gene signatures prognostic of outcome



# Hallmarks of cancer

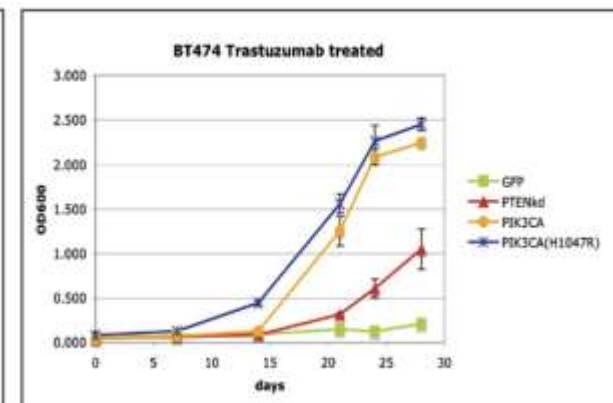
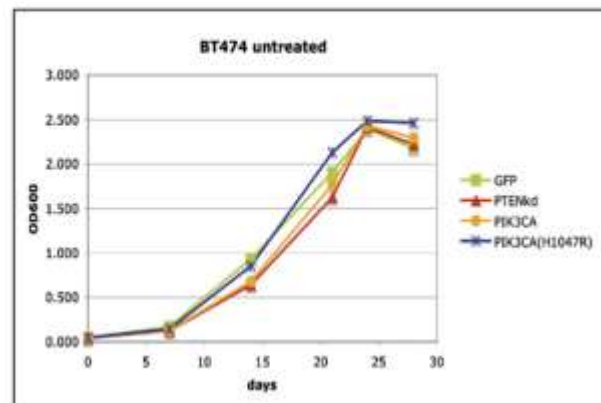
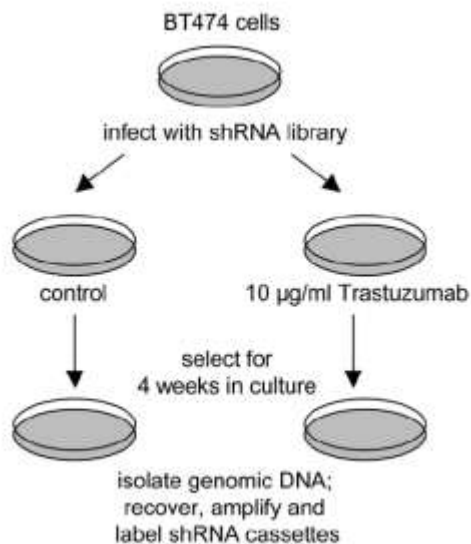
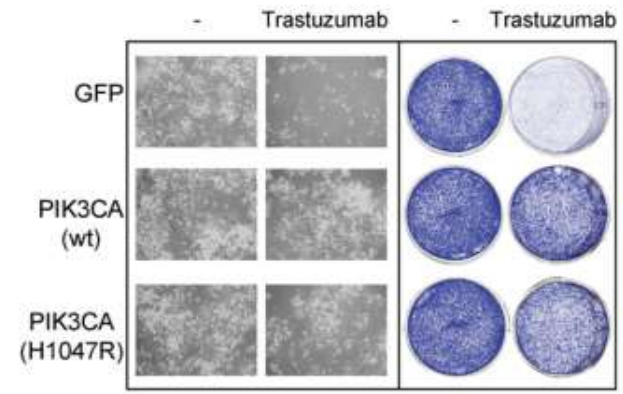
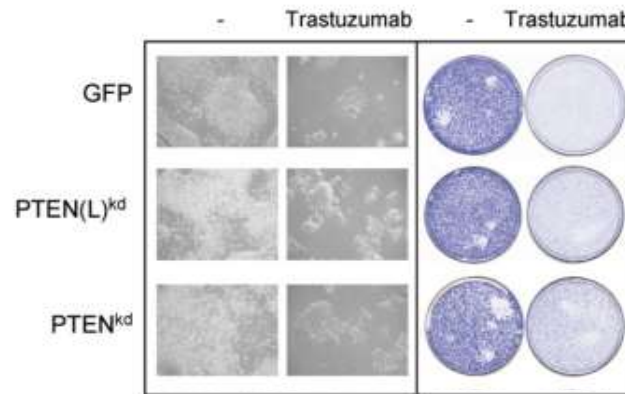
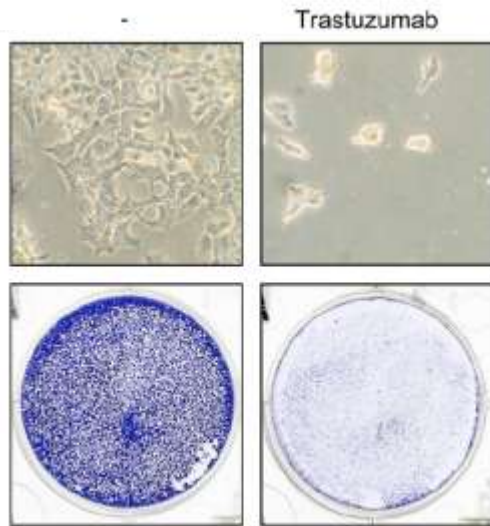


# Identification of subtypes in HER2 amplified breast cancer reveals gene signatures prognostic of outcome



# Sensitivity to HER2 therapies:

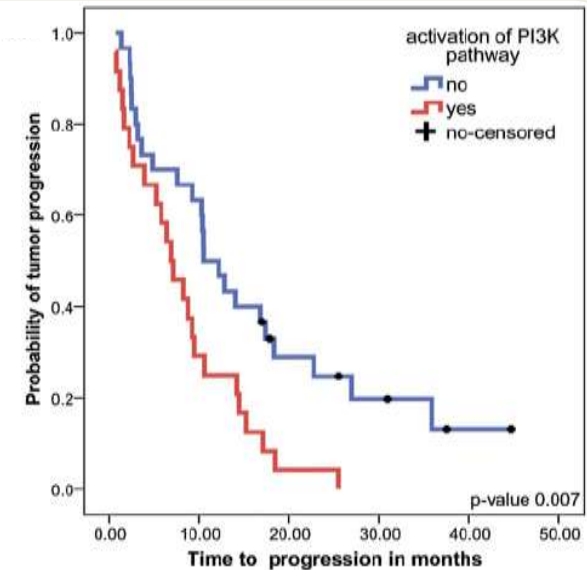
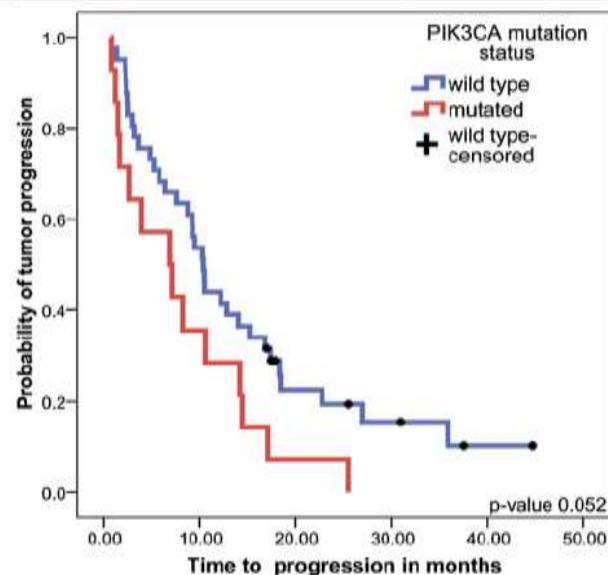
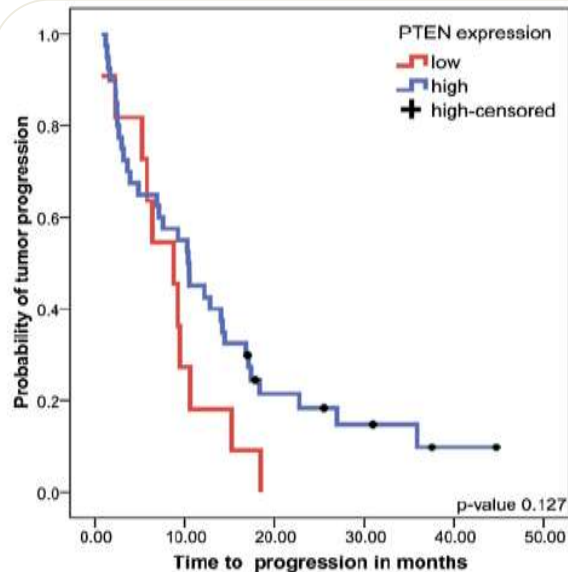
PI3K pathway is a major determinant of trastuzumab resistance in human breast cancer





# Sensitivity to HER2 therapies:

PI3K pathway is a major determinant of trastuzumab resistance in human breast cancer



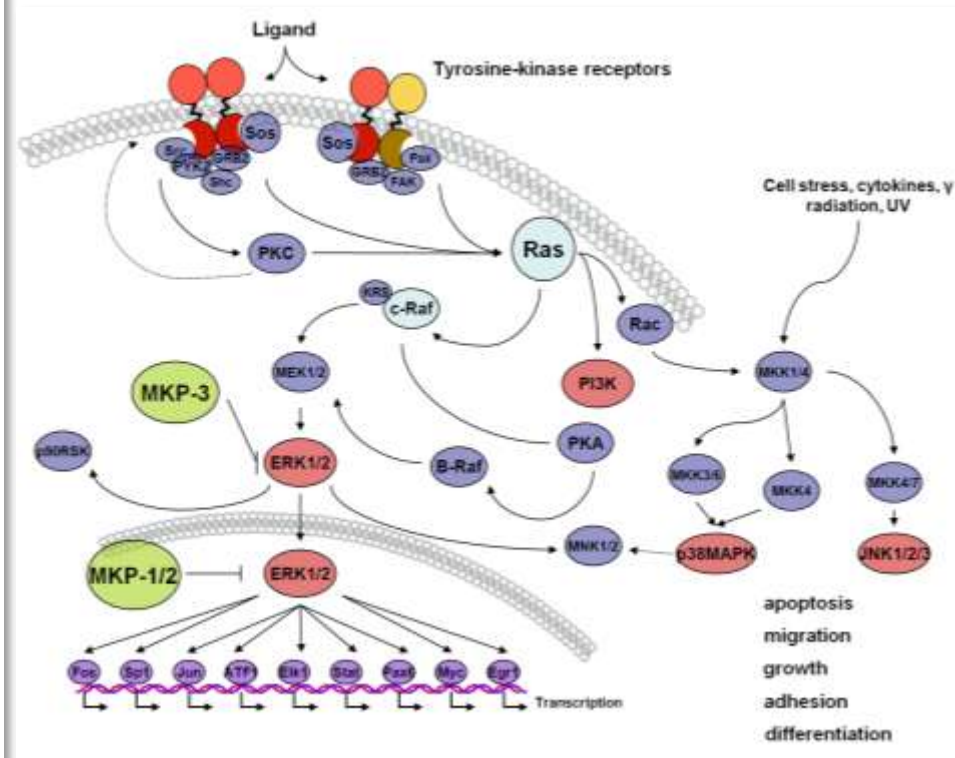
**Table 1. Multivariate Cox Regression Analysis: Individual and Joint Effects of PTEN Expression and PIK3CA Mutation on Time to Progression**

	n (Patients)	n (Events)	HR <sup>a</sup>	95% CI	p
PTEN high	39	34	1.0		
PTEN low	11	11	1.5	0.7–3.3	0.300
PIK3CA WT	40	34	1.0		
PIK3CA mutant	14	14	1.6	0.8–3.3	0.210
Not-activated PI3K pathway	29	24	1.0		
Activated PI3K pathway	24	24	1.9	1.0–3.6	0.048

HR, hazard ratio; CI, confidence interval; WT, wild-type; Not-activated PI3K pathway, PTEN high + PIK3CA wild-type; Activated PI3K pathway, PTEN low or PIK3CA mutant.

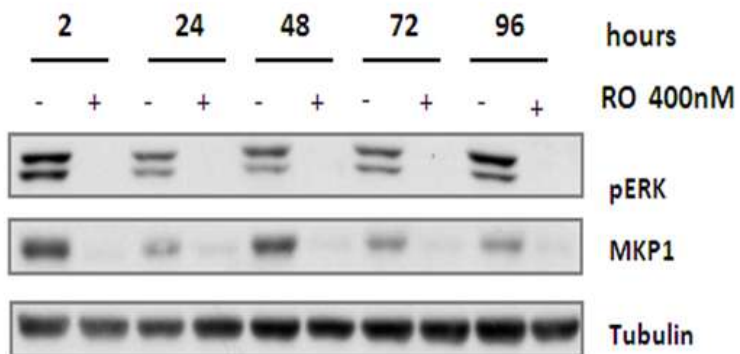
<sup>a</sup>Based on Cox regression with age as time scale, stratified for center, and adjusted for ER status.

# ERK signaling is differentially regulated in HER2 breast cancer tumors

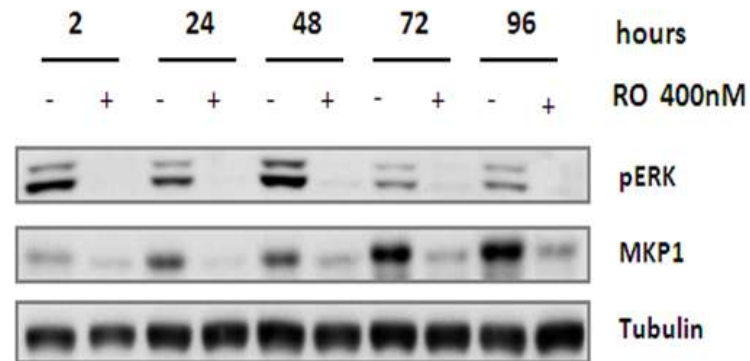


# ERK signaling inhibition potentiates HER2-therapy effects in breast cancer

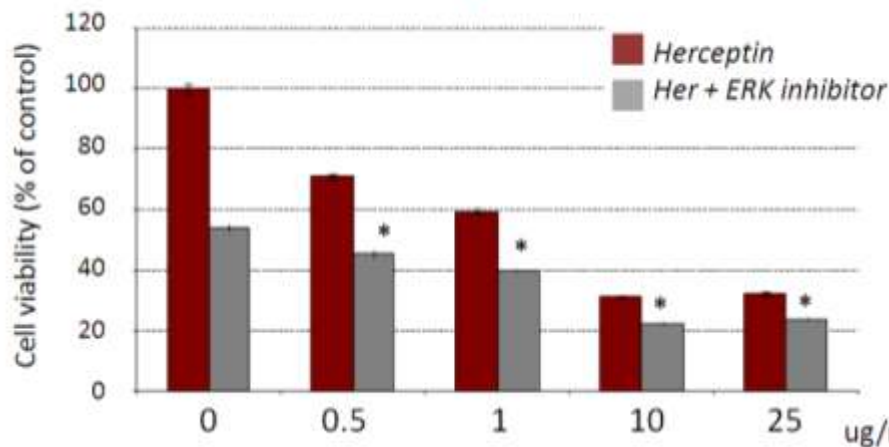
BT474



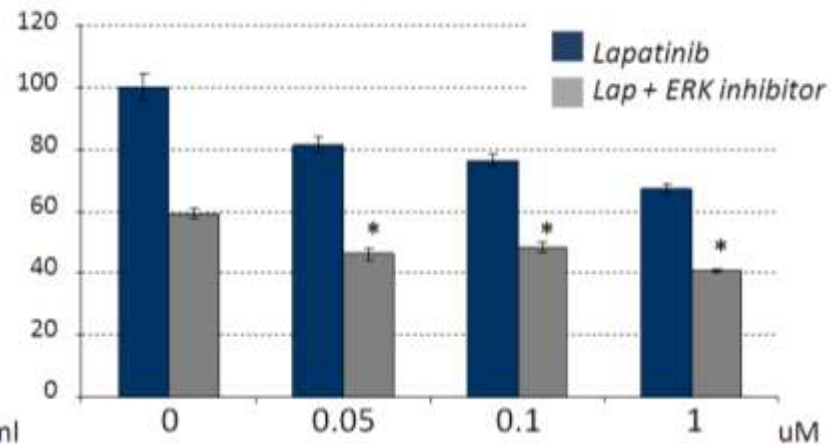
SKBR3



Herceptin

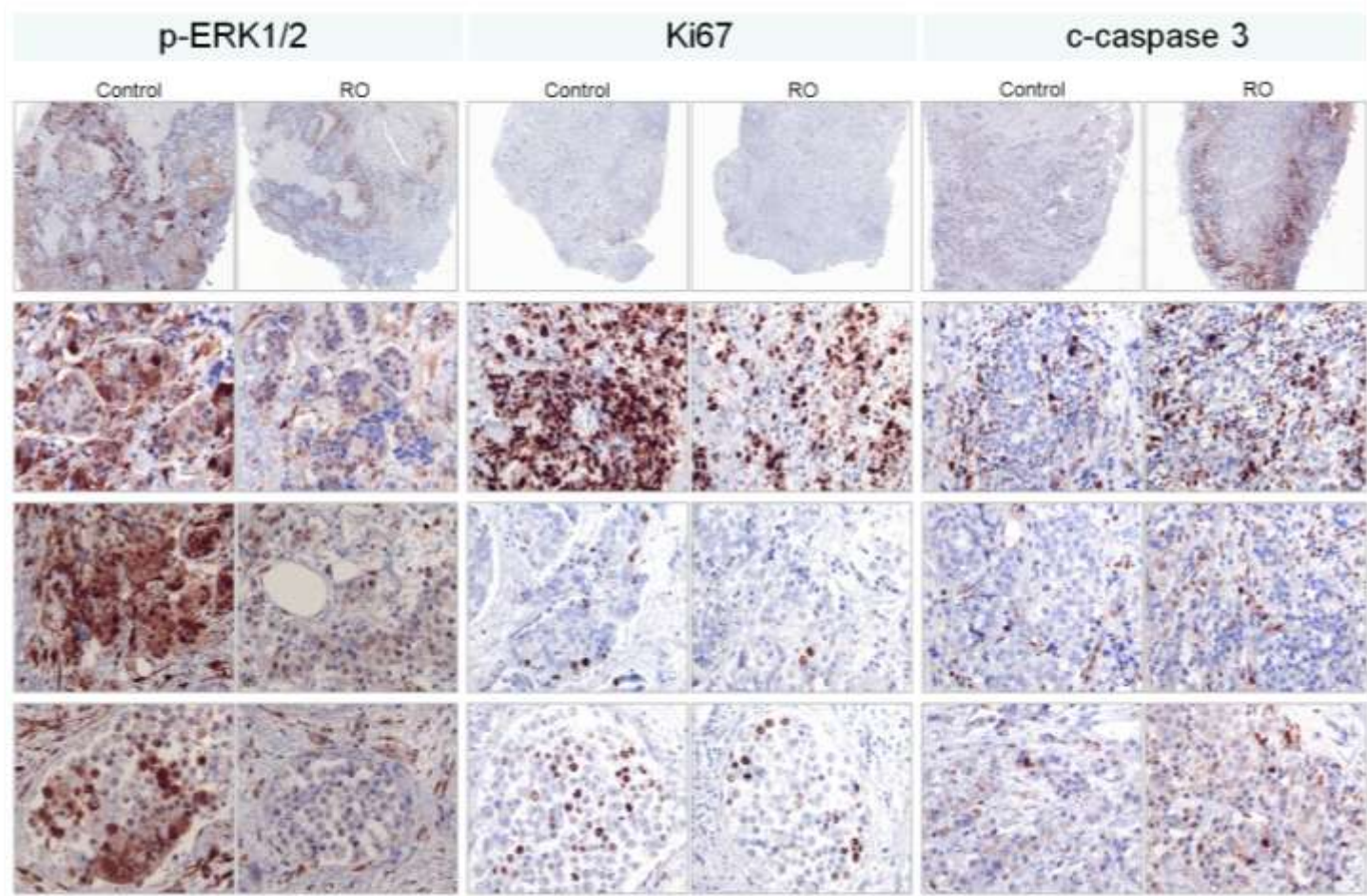


Lapatinib





# ERK signaling inhibition induces apoptosis and cell cycle stop in breast cancer tumors



pERK  
Ki67  
c-caspase 3

downregulation upregulation



# ¿Son iguales todos los carcinomas de mama HER2+?

- *Existe una alta heterogeneidad clínica, fenotípica y molecular en los carcinomas de mama HER2+*
- *La amplificación de HER2 se asocia a inestabilidad cromosómica, y, con frecuencia, a una alteración de otros oncogenes o vías de señalización intracelular (conveniencia oncogénica)*
- *Se está trabajando en la identificación de nuevas dianas terapéuticas en cáncer de mama como co-tratamiento contra HER2*