SUPPLEMENTARY MATERIALS

Supplementary Methods

Tumor samples and Gene expression

A section of the formalin-fixed paraffin-embedded (FFPE) breast tissue was first examined with haematoxylin and eosin staining to confirm presence of invasive tumor cells (≥10%) and determine the minimum tumor surface area (>4 mm2). For RNA purification (Roche® High Pure FFPET RNA isolation kit), ≥1-5 10 μm FFPE slides were used for each tumor specimen, and macrodissection was performed, when needed, to avoid normal contamination. A minimum of ~100 ng of total RNA was used to measure the expression of the 50 PAM50 subtype predictor genes, including ERBB2, and 5 housekeeping genes using the nCounter platform (Nanostring Technologies, Seattle, WA, US). Data were log base 2 transformed and normalized.

Intrinsic subtyping (Luminal A, Luminal B, HER2-enriched, Basal-like and Normal-like) was performed using the research-based PAM50 intrinsic subtype predictor as previously described [1-3]. If a tumor at baseline was identified as Normal-like despite meeting the minimum tissue criteria, new FFPE slides were cut, RNA purified and PAM50 predictor applied. If the sample remained Normal-like, this was the final call. PAM50 subtyping and ERBB2 expression levels was performed at the Translational Genomics and Targeted Therapeutics in Solid Tumors at IDIBAPS blinded from clinical data.

Monte-carlo cross-validation

We performed cross-validation to characterize the stability and expected performance of our threshold defining rule. Monte-carlo cross-validation was performed by random sampling of subjects into training and test sets, with 2/3 of samples assigned to training and 1/3 to testing. Tertiles of ERBB2 expression were estimated from the training set and used as thresholds for "high" vs "low" expression. Odds ratios of pCR were calculated for each, and the threshold was selected that corresponds to highest OR. The selected threshold was then applied to the test set to estimate sensitivity, specificity, positive predictive value, and negative predictive value. The entire process was repeated 1000 times.

References

1. Llombart-Cussac A, Cortes J, Pare L, *et al.* HER2-enriched subtype as a predictor of pathological complete response following trastuzumab and lapatinib without chemotherapy in early-stage HER2-positive breast cancer (PAMELA): an open-label, single-group, multicentre, phase 2 trial. Lancet Oncol 2017;18(4):545-554.

2. Vidal M, Peg V, Galvan P, *et al.* Gene expression-based classifications of fibroadenomas and phyllodes tumours of the breast. Mol Oncol 2015;9(6):1081-90.

3. Prat A, Cheang MC, Galvan P, *et al.* Prognostic Value of Intrinsic Subtypes in Hormone Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib. JAMA Oncol 2016;2(10):1287-1294.

SUPPLEMENTARY TABLES

Supplementary Table 1. Patient demographics of the PER-ELISA cohort*

Variables	PAM50 population N (%)	Original population N (%)
N	40	44
Age, mean y (SD)	64.2 (9.1)	65.5 (9.3)
Menopausal status		
Pre-menopausal	0	0
Post-menopausal	40 (100.0)	44 (100.0)
Tumor stage		
T1	5 (12.5)	5 (11.4)
T2	32 (80.0)	36 (81.8)
T3	3 (7.5)	3 (6.8)
Nodal status		
Negative	26 (65.0)	29 (65.9)
Positive	14 (35.0)	15 (34.1)
Missing	0 (0.0)	0 (0.0)
Hormone receptor		
Positive	40 (100.0)	44 (100.0)
Negative	0 (0.0)	0 (0.0)
PAM50		
Luminal A	7 (17.5)	NA
Luminal B	18 (45.0.)	NA
HER2-E	11 (27.5)	NA
Basal-like	0 (0.0)	NA
Normal-like	4 (10.0)	NA
pCR rate in the breast/axilla	9 (22.5)	9 (20.5)

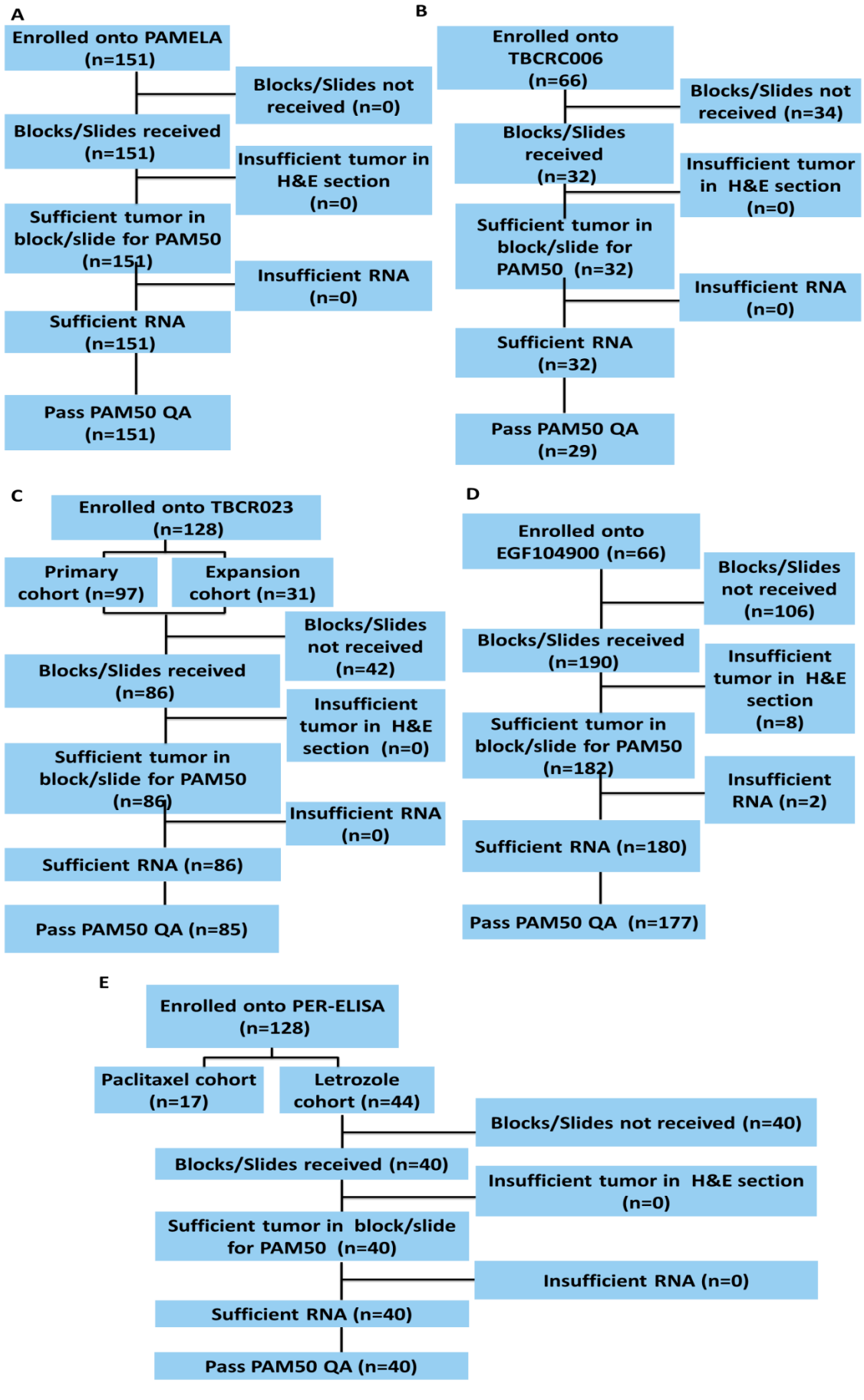
* pCR = pathological complete response; NA = not applicable; SD = standard deviation

Supplementary Table 2. Patient demographics of the EGF104900 cohort*

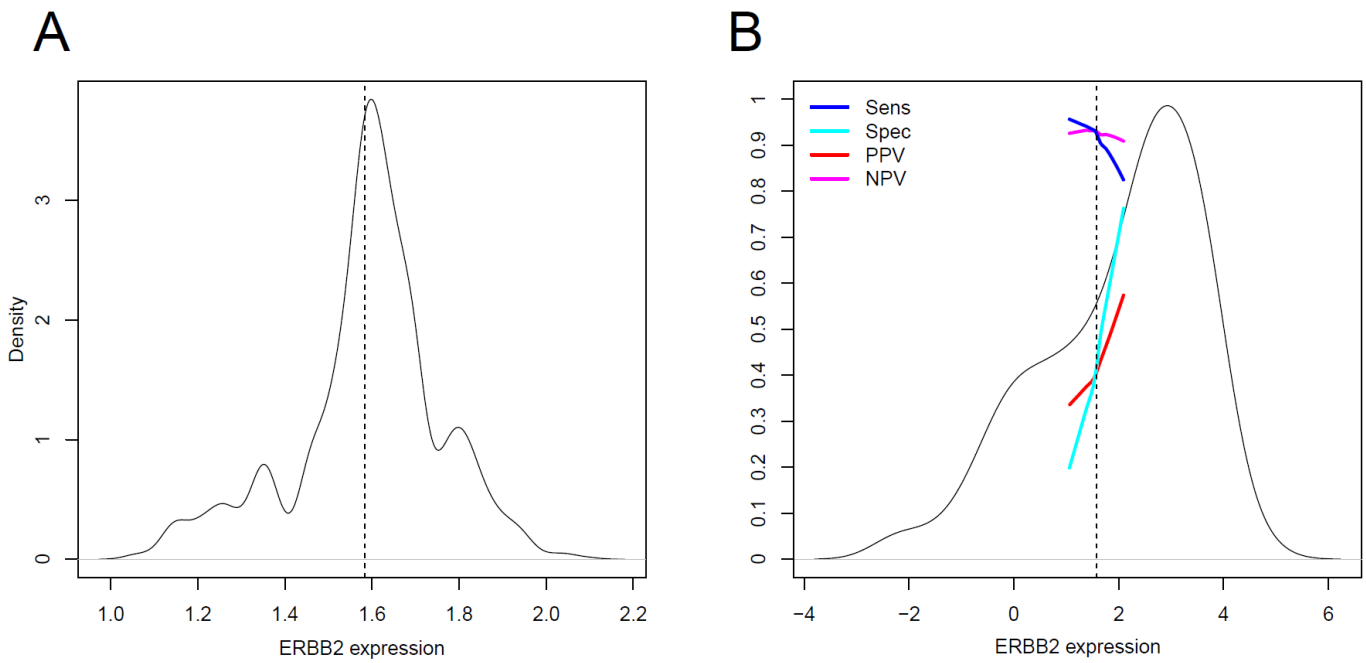
Characteristic	PAM50 polpulation		Original Population	
	Lapatinib	Lapatinib + Trastuzumab	Lapatinib	Lapatinib + Trastuzumab
N	86 (48.6)	91 (51.4)	148 (50.0)	148 (50.0)
Age (SD)	50.3 (9.5)	52.9 (11.2)	51	52
ECOG				
0	38 (43.0)	40 (44.0)	69 (46.6)	80 (54.1)
1	37 (43.0)	40 (44.0)	73 (49.3)	61(41.2)
2	4 (4.7)	2 (2.2)	6 (4.1)	7 (4.7)
Missing	8 (9.3)	9 (9.9)	0 (0.0)	0 (0.0)
Hormone receptor				
Positive	44 (51.2)	42 (46.2)	73 (49.3)	73 (49.3)
Negative	41(47.7)	47 (51.6)	75 (50.7)	75 (50.7)
Missing	1 (1.2)	2 (2.2)	0 (0.0)	0 (0.0)
Visceral disease				
Yes	63 (73.3)	63 (69.2)	110 (74.3)	105 (70.9)
No	23 (26.7)	28 (30.8)	38 (25.7)	43 (29.1)
PAM50				
Luminal A	2 (2.3)	2 (2.2)	NA	NA
Luminal B	7 (8.1)	11 (12.1)	NA	NA
HER2-E	52 (60.5)	51 (56.0)	NA	NA
Basal-like	13 (15.1)	14 (15.4)	NA	NA
Normal-like	12 (14.0)	13 (14.3)	NA	NA

* NA = not applicable; SD = standard deviation

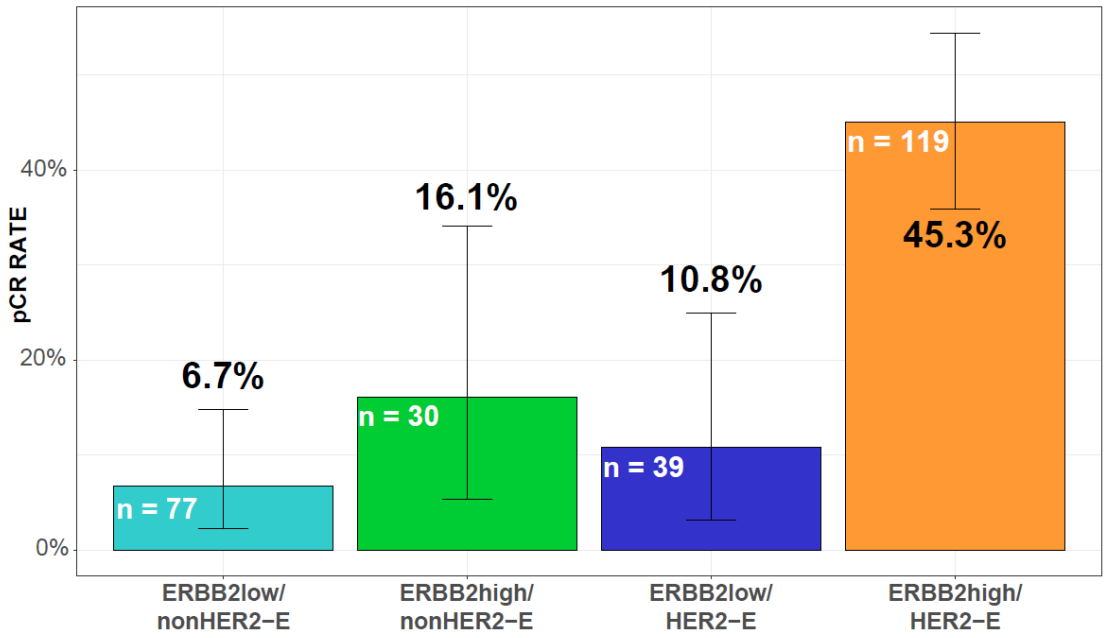
SUPPLEMENTARY FIGURES



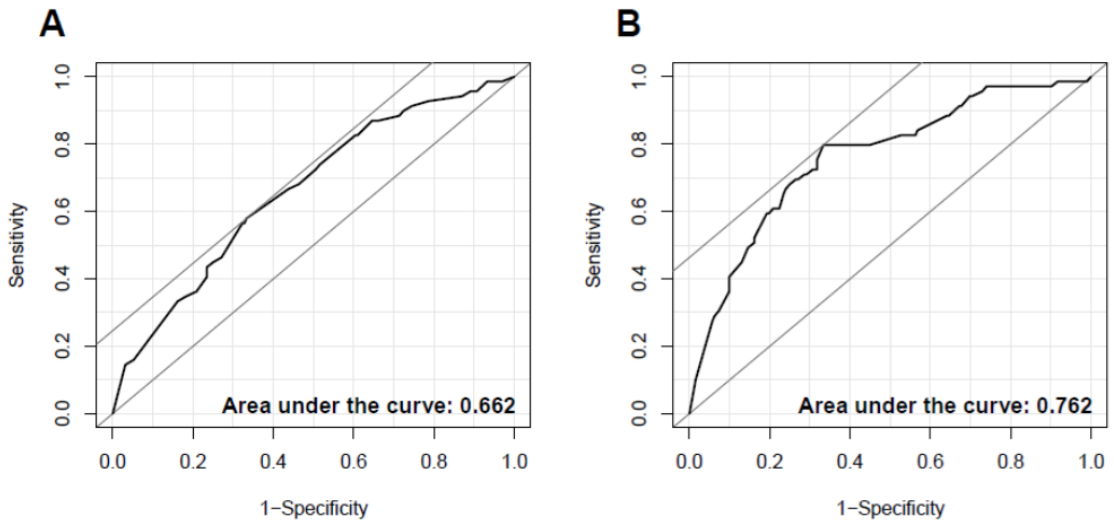
Supplementary Figure 1. **CONSORT diagram for the availability of samples for analysis from the 5 clinical trials. (A)** Pamela trial **(B)** TBCRC 006 trial **(C)** TBCRC 023 trial **(D)** EGF104900 trial **(E)** PER-ELISA Trial**.** H&E = hematoxylin-eosin staining**;** QA = quality assurance.



Supplementary Figure 2. Stability and performance of the ERBB2 cutpoint identified in the SOLTI-PAMELA cohort. (**A**) Distribution of thresholds chosen (solid line) during cross-validation are centered near the final value selected from the complete training set (dotted line). (**B**) Distribution of ERBB2 expression (solid black line) with summarized performance estimates for all selected thresholds (colored lines). Sens = sensitivity; Spec = specificity; PPV = Positive predictive values; NPV = negative predictive values.

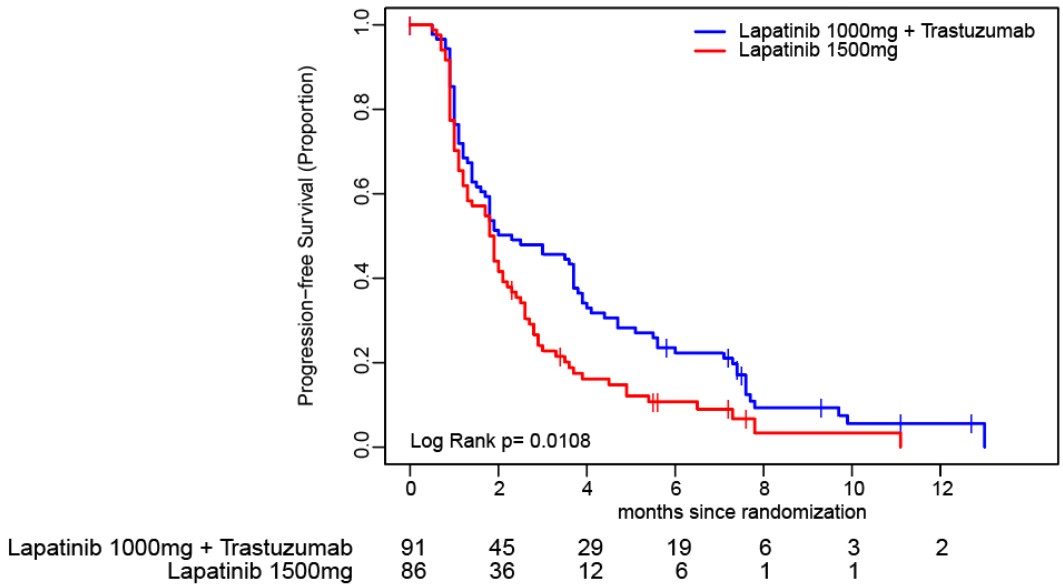


Supplementary Figure 3. Pathologic complete response according to ERBB2 mRNA levels and HER2-E subtype. Erros bars represent the 95% confidence intervals of pCR rate. pCR = pathological complete response

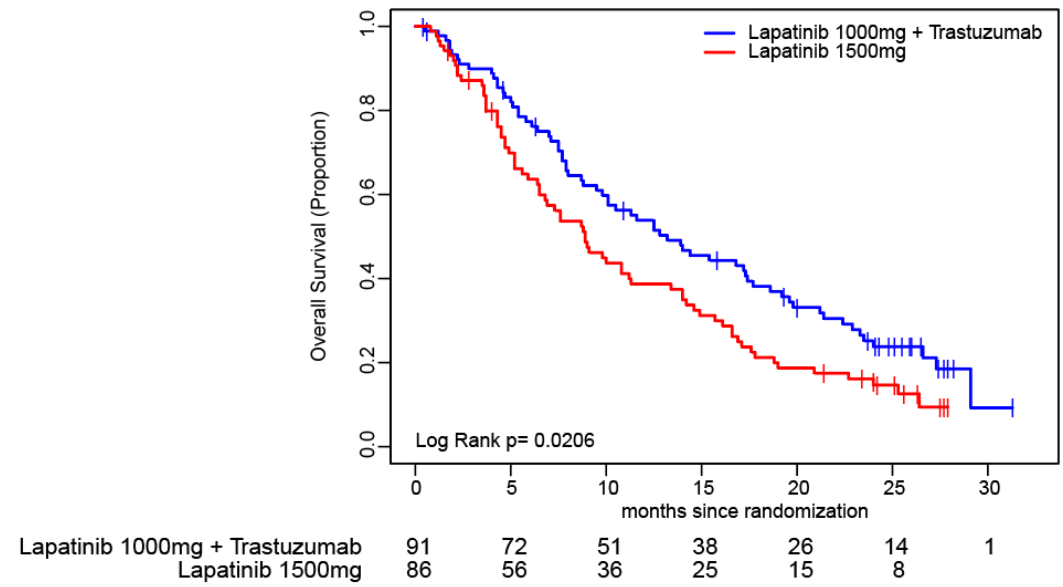


Supplementary Figure 4: Predictive performance of variables in the combined neoadjuvant HER2+ cohort. (A) All clinical-pathological variables (tumor stage, menopausal status, treatment duration, hormone receptor status, nodal status and cohort). (B) All clinical-pathological variables and HER2-E/ERBB2-high biomarker.

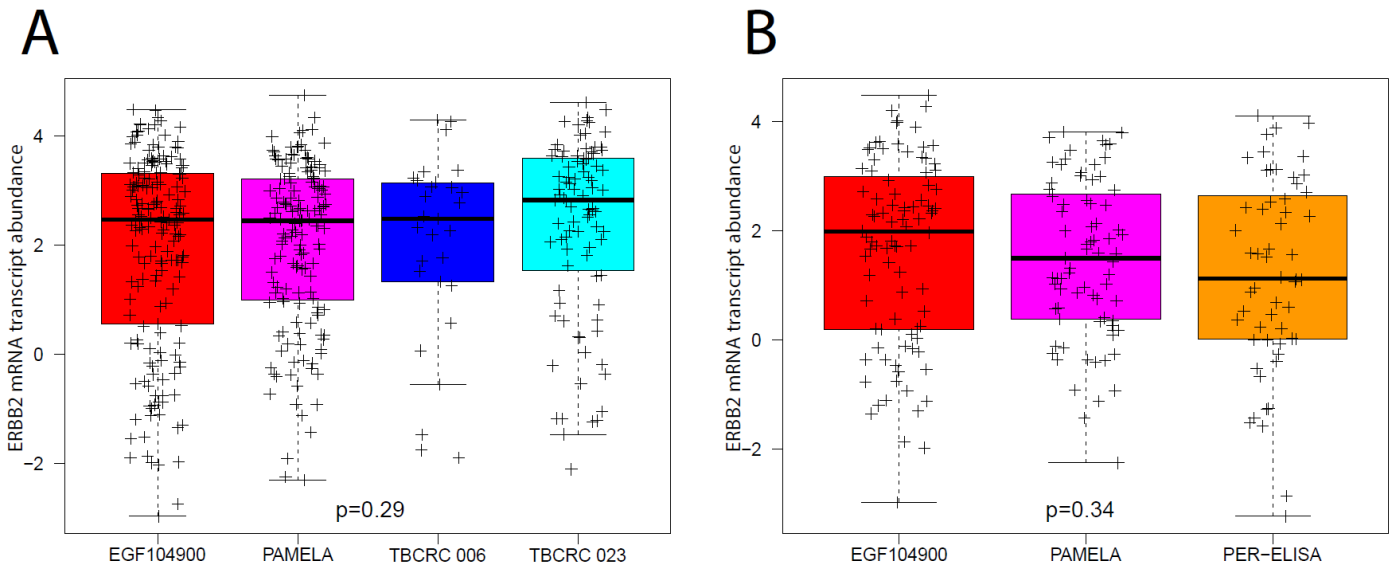
A



B



Supplementary Figure 5. **Progression-free survival (PFS) and overall survival (OS) curves in PAM50 population of the EGF104900 trial based on treatment.** (**A**) Progression-free survival; (**B**) Overall survival. Estimates of PFS and OS were from Kaplan–Meier curves and tests of differences by log-rank test.



Supplementary Figure 6. Expression of ERBB2 mRNA across studies. (**A**) Cohorts with hormone receptor-positive and -negative tumors. (**B**) Cohorts with hormone receptor-positive disease-only. Error bars represent the range of variation. Tests of differences by Analysis of Variance test. The significance level was set to a two-sided α of 0.05.