

# A PAM50-Based Chemoendocrine Score for Hormone Receptor–Positive Breast Cancer with an Intermediate Risk of Relapse

Aleix Prat<sup>1,2,3</sup>, Ana Lluch<sup>4</sup>, Arran K. Turnbull<sup>5</sup>, Anita K. Dunbier<sup>6</sup>, Lourdes Calvo<sup>7</sup>, Joan Albanell<sup>8</sup>, Juan de la Haba-Rodríguez<sup>9</sup>, Angels Arcusa<sup>10</sup>, José Ignacio Chacón<sup>11</sup>, Pedro Sánchez-Rovira<sup>12</sup>, Arrate Plazaola<sup>13</sup>, Montserrat Muñoz<sup>1,2</sup>, Laia Paré<sup>3</sup>, Joel S. Parker<sup>14</sup>, Nuria Ribelles<sup>15</sup>, Begoña Jimenez<sup>15</sup>, Abdul Aziz Bin Aiderus<sup>5</sup>, Rosalía Caballero<sup>16</sup>, Barbara Adamo<sup>1,2</sup>, Mitch Dowsett<sup>17</sup>, Eva Carrasco<sup>16</sup>, Miguel Martín<sup>18</sup>, J. Michael Dixon<sup>5</sup>, Charles M. Perou<sup>14,19,20</sup>, and Emilio Alba<sup>15</sup>

## Abstract

**Purpose:** Hormone receptor–positive (HR<sup>+</sup>) breast cancer is clinically and biologically heterogeneous, and subgroups with different prognostic and treatment sensitivities need to be identified.

**Experimental Design:** Research-based PAM50 subtyping and expression of additional genes was performed on 63 patients with HR<sup>+</sup>/HER2<sup>−</sup> disease randomly assigned to neoadjuvant multiagent chemotherapy versus endocrine therapy in a phase II trial. The biology associated with treatment response was used to derive a PAM50-based chemoendocrine score (CES). CES's predictive ability was evaluated in 4 independent neoadjuvant data sets ( $n = 675$ ) and 4 adjuvant data sets ( $n = 1,505$ ). The association of CES, intrinsic biology, and PAM50 risk of relapse (ROR) was explored across 6,007 tumors.

**Results:** Most genes associated with endocrine sensitivity were also found associated with chemotherapy resistance. In the chemotherapy test/validation data sets, CES was independently asso-

ciated with pathologic complete response (pCR), even after adjusting for intrinsic subtype. pCR rates of the CES endocrine-sensitive (CES-E), uncertain (CES-U), and chemotherapy-sensitive (CES-C) groups in both data sets combined were 25%, 11%, and 2%, respectively. In the endocrine test/validation data sets, CES was independently associated with response. Compared with ROR, >90% of ROR-low and ROR-high tumors were identified as CES-E and CES-C, respectively; however, each CES group represented >25% of ROR-intermediate disease. In terms of survival outcome, CES-C was associated with poor relapse-free survival in patients with ROR-intermediate disease treated with either adjuvant endocrine therapy only or no adjuvant systemic therapy, but not in patients treated with (neo)adjuvant chemotherapy.

**Conclusions:** CES is a genomic signature capable of estimating chemoendocrine sensitivity in HR<sup>+</sup> breast cancer beyond intrinsic subtype and risk of relapse. *Clin Cancer Res*; 1–10. ©2016 AACR.

## Introduction

Approximately 70% of invasive breast cancers at diagnosis are hormone receptor–positive and HER2-negative (HR<sup>+</sup>/HER2<sup>−</sup>; ref. 1, 2). However, HR<sup>+</sup>/HER2<sup>−</sup> disease is clinically and biologically heterogeneous and further subclassifica-

tions are needed to better tailor current and future treatments (3–5).

Over the last decade, molecular characterization studies have identified and extensively investigated the two main molecular subtypes within HR<sup>+</sup>/HER2<sup>−</sup> disease (i.e., Luminal A and B;

<sup>1</sup>Department of Medical Oncology, Hospital Clínic i Provincial, Barcelona, Spain. <sup>2</sup>Translational Genomics and Targeted Therapeutics in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain. <sup>3</sup>Translational Genomics Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain. <sup>4</sup>Department of Medical Oncology, Valencia University Hospital, Valencia, Spain. <sup>5</sup>University of Edinburgh Cancer, Research UK Centre, Edinburgh. <sup>6</sup>Department of Biochemistry, University of Otago, Dunedin, New Zealand. <sup>7</sup>Department of Medical Oncology, A Coruña University Hospital Complex, A Coruña, Spain. <sup>8</sup>Department of Medical Oncology, Hospital del Mar Medical Research Institute-IMIM and Pompeu Fabra University, Barcelona, Spain. <sup>9</sup>Department of Medical Oncology, Biomedical Research Institute-IMIBIC, Reina Sofía Hospital Complex, Córdoba, Spain. <sup>10</sup>Department of Medical Oncology, Consorci Sanitari de Terrassa, Barcelona, Spain. <sup>11</sup>Department of Medical Oncology, Virgen de la Salud Hospital, Toledo, Spain. <sup>12</sup>Department of Medical Oncology, Jaén Hospital Complex, Jaén, Spain. <sup>13</sup>Department of Medical Oncology, Onkologikoa, Donostia, Spain. <sup>14</sup>Lineberger Comprehensive Cancer Center, University of North Car-

olina, Chapel Hill, North Carolina. <sup>15</sup>Department of Medical Oncology, Virgen de la Victoria University Hospital, Málaga, Spain. <sup>16</sup>GEICAM (Spanish Breast Cancer Research Group), Madrid, Spain. <sup>17</sup>Academic Department of Biochemistry, Royal Marsden Foundation Trust, London, United Kingdom. <sup>18</sup>Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense, Madrid, Spain. <sup>19</sup>Department of Genetics, University of North Carolina, Chapel Hill, North Carolina. <sup>20</sup>Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina.

**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

**Corresponding Author:** Aleix Prat, Department of Medical Oncology, Hospital Clínic de Barcelona, Casanova 170, 08036, Barcelona, Spain. Phone: 646847709; Fax: 34932100860; E-mail: [alprat@clinic.ub.es](mailto:alprat@clinic.ub.es)

**doi:** 10.1158/1078-0432.CCR-16-2092

©2016 American Association for Cancer Research.

## Translational Relevance

Hormone receptor–positive (HR<sup>+</sup>) breast cancer is clinically and biologically heterogeneous, and subgroups with different prognostic and treatment sensitivities need to be identified. Here, we present the development and clinical validation across multiple studies of a gene expression–based predictor, based on the well-known PAM50 assay, which is associated with chemotherapy and endocrine therapy response in early breast cancer beyond PAM50 risk of relapse (ROR) and intrinsic subtypes. The potential clinical utility of this PAM50-based chemoendocrine score (CES) predictor might be in the PAM50 ROR intermediate, where the proportion of each CES group (endocrine-sensitive, intermediate, and chemosensitive) is more than 25%. This is important as we await the results of large adjuvant clinical trials, such as TailorX or RxPonder, that have randomly assigned patients with intermediate risk to adjuvant chemotherapy or not.

refs. 1, 2, 6). Luminal A tumors have an improved prognosis at 5- and 10-year follow-up compared with Luminal B tumors irrespective of classical clinical–pathologic variables (e.g., tumor size and nodal status) and (neo)adjuvant treatment (i.e., endocrine and chemotherapy; refs. 1, 2, 7, 8). In terms of treatment sensitivity, Luminal A tumors achieve significant lower rates of pathologic complete response (pCR) than Luminal B tumors following neoadjuvant multiagent chemotherapy (9–12). However, less clear is the difference in endocrine sensitivity between the two luminal subtypes (13, 14).

Today, adjuvant endocrine therapy for 5 to 10 years is recommended for all patients with HR<sup>+</sup>/HER2<sup>−</sup> early breast cancer, whereas chemotherapy is recommended for patients with intermediate- and high-risk tumors (15). However, the relationship between therapy and risk warrants further study considering that risk is associated with both factors related to tumor biology and clinical–pathologic features such as tumor size and nodal status, whereas therapy responsiveness is generally considered to be independent of clinical–pathologic factors.

## Materials and Methods

### GEICAM/2006-03 clinical trial

Pretreatment core biopsy samples from patients recruited in the luminal cohort of the GEICAM/2006-03 phase II neoadjuvant clinical trial were evaluated (16). In this study, 95 patients with estrogen receptor (ER)–positive (Allred 3–8), progesterone receptor (PR)–positive (Allred 3–8), HER2<sup>−</sup> (according to the ASCO/CAP guidelines; ref. 17), and cytokeratin 8/18-positive breast cancer were randomly assigned to receive 24 weeks of neoadjuvant chemotherapy or endocrine therapy. Chemotherapy consisted of epirubicin combined with cyclophosphamide for 4 cycles followed by docetaxel for 4 cycles. Endocrine therapy consisted of oral exemestane. Premenopausal patients received goserelin for 6 doses.

### GEICAM 2006-03 pathologic response endpoint

The 5-point scale Miller and Payne histologic grading system (18) was used to measure tumor response. In this study, the Miller

and Payne scale was reduced to a 3-point scale in order to have a fair number of cases in each category and arm: no response (grades 1 and 2), intermediate response (grade 3), and high response (grades 4 and 5).

### GEICAM 2006-03 gene expression analysis

Sixty-three of 95 pretreatment tumor samples were available for gene expression analyses. Total RNA was purified to measure the expression of 543 breast cancer–related genes, 5 housekeeping genes and 14 negative and positive controls using the nCounter platform (Nanostring Technologies; ref. 19). Raw gene expression can be found in Supplementary Material.

### Independent/testing data sets

Gene expression and response data were evaluated from 4 independent neoadjuvant data sets (Supplementary Material; refs. 14, 20–24). Gene expression and survival data were evaluated from 4 independent data sets of patients with early breast cancer (Supplementary Material; refs. 2, 20, 25, 26).

### Intrinsic subtype assignment

All tumors were assigned to an intrinsic molecular subtype of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like) and the normal-like group using the research-based PAM50 subtype predictor (27, 28), except for the Malaga cohort where the PAM50 standardized and commercial nCounter-based assay was used. Before subtyping, each individual dataset was normalized accordingly as previously reported (29), except for the Malaga cohort that was normalized by Nanostring according to their algorithm. Of note, the Edinburgh microarray-based dataset is composed of ER<sup>+</sup> samples-only and proper centering for intrinsic subtyping calling was not possible (30). In this dataset, CES was evaluated as a continuous variable since it is not affected by centering.

### Combined cohort of primary breast cancer

To evaluate the relationship between PAM50 subtype calls, prognosis (ROR-P) and CES, we combined PAM50 data from 7 independent and previously reported cohorts (1, 2, 11, 20, 31–33) representing a total of 6,007 primary tumor samples. CES was evaluated in each individual cohort, and a combined matrix was created (Supplementary Material).

### Statistical analysis

Biologic analysis of gene lists was performed with DAVID 6.7 annotation tool (34) using the 543-gene list as background. The association between the expression of each gene and Miller–Payne response (3 categories) was assessed by a quantitative Significance Analysis of Microarrays (SAM; ref. 35). In both testing data sets, association between each variable and pCR or clinical/radiological response was assessed by univariate and multivariable logistic regression analyses. The predictive performance of CES was evaluated using receiver operating characteristic (ROC) curve analysis. Estimates of survival were from the Kaplan–Meier curves and tests of differences by the log-rank test. Univariate and multivariable Cox models were used to test the independent prognostic significance of each variable. Reported *P* values are two sided.

**Table 1.** Clinical-pathologic characteristics and subtype distribution in the GEICAM 2006-03 study

	CT (%)	ET (%)	P
Number	32 (—)	31 (—)	—
Age (mean)	53.7 (—)	52.3 (—)	0.596
Menopausal status			
Premenopausal	14 (44%)	14 (45%)	1.000
Postmenopausal	18 (56%)	17 (55%)	
Tumor stage			
T1	1 (3%)	2 (6%)	0.420
T2	23 (72%)	25 (81%)	
T3	8 (25%)	4 (13%)	
Mean tumor size (cm)	4.2	3.8	0.278
Node			
N0	15 (47%)	19 (61%)	0.501
N1	16 (50%)	11 (35%)	
N2	1 (3%)	1 (3%)	
Grade			
G1	0 (0%)	0 (0%)	0.862
G2	8 (25%)	6 (19%)	
G3	18 (56%)	19 (61%)	
G4	6 (19%)	6 (19%)	
Histologic type			
Ductal	26 (81%)	26 (84%)	1.000
Lobular	2 (6%)	2 (6%)	
Others	4 (13%)	3 (10%)	
Ki-67 IHC (mean)	31.1	33.5	0.720
Miller-Payne response (mean)	2.6	2.2	0.124
PAM50			
Luminal A	16 (50%)	13 (42%)	0.564
Luminal B	11 (34%)	13 (42%)	
HER2-E	0 (0%)	1 (3%)	
Basal-like	2 (6%)	0 (0%)	
Normal-like	3 (9%)	4 (13%)	

Abbreviations: CT, chemotherapy arm; ET, endocrine therapy arm.

## Results

### GEICAM 2006-03 dataset

Sixty-three pre- and postmenopausal patients were evaluated in this study (Table 1). Most patients presented ductal carcinomas (83%), tumor sizes of 2 to 5 cm (76%), histologic grade 3 tumors (59%), clinical node-negative disease (54%), and luminal disease by PAM50 (84%).

Following chemotherapy, Luminal B tumors showed higher Miller-Payne response than Luminal A disease (mean 2.0 vs. 1.4,  $P = 0.048$ ). However, no difference in response between the two luminal subtypes was observed following endocrine therapy ( $P = 0.407$ ). In addition, no statistical significant interaction ( $P = 0.429$ ) between subtype and treatment (endocrine vs. chemotherapy) for tumor response was observed. Interestingly, the only patient who achieved a pCR (i.e., Miller-Payne grade 5) had a Basal-like tumor and was contained within the chemotherapy arm.

### Gene expression association with treatment sensitivity

To understand the biology associated with either chemotherapy or endocrine sensitivity within HR<sup>+</sup>/HER2<sup>-</sup> disease, we explored the association between the expression of 543 breast cancer-related genes and Miller-Payne response in each treatment arm. High expression of 70 (12.9%) and 17 (3.1%) genes was found significantly associated ( $P < 0.05$  uncorrected for multiple comparisons) with response after endocrine therapy and chemotherapy, respectively. The gene list associated with endocrine therapy response was enriched for the following bio-

logical processes (Supplementary Material): vasculature development (e.g., AKT1 and catenin beta 1), tube development (e.g., FOXA1 and gremlin 1), and cell growth (e.g., androgen receptor and fibroblast growth factor receptor 1). On the other hand, the gene list associated with chemotherapy response was enriched for cell cycle (e.g., EXO1 and MKI67) and extracellular matrix (e.g., netrin 4 and thrombospondin 1).

We then evaluated the interaction between the expressions of each individual gene with response to therapy (endocrine vs. chemotherapy). Interestingly, 41 of the 70 genes associated with response to endocrine therapy, and 8 of 17 genes associated with chemotherapy response, showed a significant interaction with treatment ( $P < 0.05$  uncorrected for multiple comparisons). Thus, the biological factors associated with endocrine sensitivity seemed to be associated, at the same time, with chemotherapy resistance, and vice versa. Indeed, an overall inverse pattern was observed between expression of most genes and response to treatment (Fig. 1A).

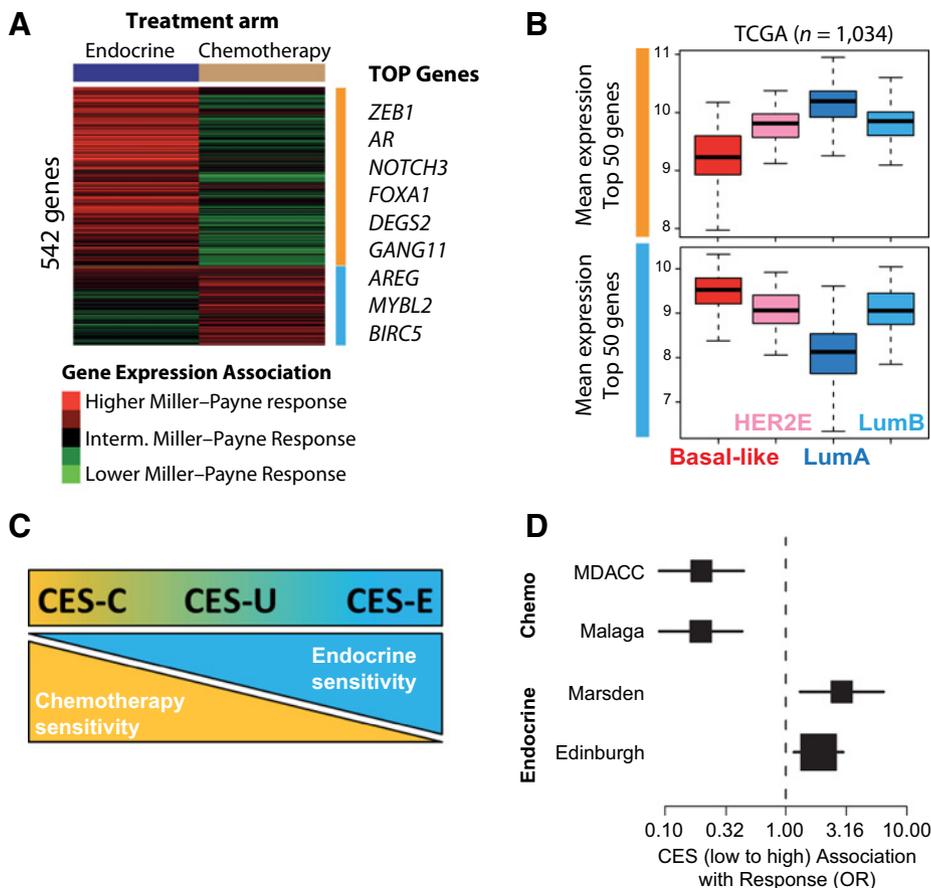
To further understand the biological factors associated with treatment response, we evaluated the mean expression of genes associated with high endocrine but low chemotherapy sensitivity, or low endocrine but high chemotherapy sensitivity, across 1,034 primary tumors representing all intrinsic molecular subtypes of breast cancer (Fig. 1B). The results revealed that the biology associated with chemoendocrine sensitivity is mostly driven by the Luminal A (i.e., high endocrine but low chemotherapy sensitive) versus Basal-like biology (i.e., low endocrine but high chemotherapy sensitive).

### Development of a PAM50-based CES

Our previous results suggested that capturing the relative differences in the Luminal A versus Basal-like biology within HR<sup>+</sup>/HER2<sup>-</sup> could help better predict endocrine and chemotherapy sensitivity. To capture this biological state in each tumor, we obtained, from the PAM50 classification algorithm, the correlation coefficients (CC) of each sample to the PAM50 Luminal A and Basal-like subtype centroids, and then subtracted the 2 values to create the chemoendocrine score (CES = CC to Luminal A - CC to Basal-like). Thus, samples with a positive score were identified as being more endocrine sensitive than chemotherapy sensitive, whereas samples with a negative score were identified as being more chemotherapy sensitive (CES-C) than endocrine sensitive (CES-E; Fig. 1C). From GEICAM 2006-03 samples, cutoffs based on tertile groups were determined [CES-E vs. CES uncertain (CES-U) group, cutoff = 0.70; CES-U vs. CES-C group, cutoff = 0.30]. The interaction of the CES score (as a continuous variable) with treatment in the GEICAM 2006-03 trial provides some evidence of association ( $P = 0.059$ ).

### MDACC-based dataset

We evaluated a combined dataset of 272 patients with HR<sup>+</sup>/HER2<sup>-</sup> disease treated with anthracycline/taxane-based neoadjuvant chemotherapy across several neoadjuvant trials (Fig. 1D and Table 2). In this dataset, 51.5%, 25.8%, and 22.7% of the samples were identified as CES-E, -U, and -C, respectively. The rates of pCR across the CES-E, -U, and -C groups were 2.4%, 9.0%, and 23.7%, respectively ( $P < 0.0001$ ), and were found to be similar even if non-luminal tumors were removed (2.2%, 8.8%, and 25.0%). The neoadjuvant chemotherapy predictive ability of CES was independent of clinical-pathologic variables and intrinsic subtype (Table 3; Supplementary Table S1). Similar results were

**Figure 1.**

Gene expression association with either chemotherapy or endocrine therapy sensitivity. **A**, Association between the expression of each individual gene ( $n = 542$ ) and Miller-Payne response in each arm of the GEICAM 2006-03 trial. Selected top genes whose expression is found significantly associated with response are shown on the right. **B**, Mean expression of the top 50 genes associated with endocrine sensitivity (top) and chemotherapy sensitivity (bottom) in the GEICAM 2006-03 trial across the intrinsic subtypes of breast cancer. The RNAseq-based gene expression data have been obtained from The Cancer Genome Atlas breast cancer project data portal (<https://tcga-data.nci.nih.gov/tcga/>). **C**, Significance and scoring of the CES. **D**, Association of CES (as a continuous variable) with chemotherapy or endocrine therapy response in the 4 validation data sets.

obtained when residual cancer burden (RCB) was used as the endpoint (Supplementary Tables S2 and S3).

Six gene expression-based signatures (i.e., PAM50 proliferation score, ROR-P, genomic grade index, SET index, chemopredictor, DLDA30, and RCB predictor) have been previously reported in this dataset (20). In addition, we applied a microarray-based version of OncotypeDX Recurrence Score (36, 37). Here, we evaluated the performance of CES to predict pCR within HR<sup>+</sup>/HER2<sup>-</sup> disease compared with these 7 gene signatures. Interestingly, CES provided the highest aROC (Supplementary Tables S4–S12) either as a continuous variable (aROC = 0.770) or as group categories (aROC = 0.765). The second most predictive signature was the RCB predictor (aROC = 0.740). Of note, the RCB predictor was trained using 165 of 272 (60.7%) HR<sup>+</sup>/HER2<sup>-</sup> samples from this dataset (i.e., the training dataset). When these training samples were removed, CES showed a higher performance either as a continuous variable (aROC = 0.805) or as group categories (aROC = 0.786) than the RCB predictor (aROC = 0.640).

#### Malaga-based dataset

We evaluated a dataset of 180 patients with HR<sup>+</sup>/HER2<sup>-</sup> disease treated with anthracycline/taxane-based neoadjuvant chemotherapy (Table 2). In this dataset, 46.1%, 16.1%, and 37.8% of the samples were identified as CES-E, -U, and -C, respectively. The pCR and RCB 0/1 rates across the CES-E, -U, and -C groups were 2.4%/9.6%, 3.4%/17.2%, and 13.2%/30.9%, respectively ( $P = 0.022$  and  $0.004$ ).

To test the ability of CES to predict chemotherapy response independently of known clinical-pathologic variables and intrinsic subtype, we performed a multivariable logistic regression analysis using RCB (0/1 vs. 2/3) as the endpoint since only 12 samples achieved a RCB 0 (i.e., pCR) in this dataset. The results revealed that CES provided independent predictive information beyond intrinsic subtype (Table 4), Ki-67 by IHC (Supplementary Table S13) and PAM50 ROR score (Supplementary Table S14). The aROC of CES for predicting RCB 0/1 was 0.746. Finally, we observed a significant association between CES and Miller-Payne response data (Supplementary Fig. S1).

#### Marsden-based dataset: CES and endocrine sensitivity

We evaluated a dataset of 103 postmenopausal patients with HR<sup>+</sup> disease treated with anastrozole for 16 weeks in the neoadjuvant setting (Table 2). In this dataset, 23.5%, 34.3%, and 42.2% of samples were identified as CES-E, -U, and -C, respectively. Clinical tumor response (complete and partial response versus stable and progressive disease) was used as the endpoint. No pCR was observed in this dataset. The rates of clinical tumor response across the CES-E, -U, and -C groups were 75.0%, 48.6%, and 44.2%, respectively ( $P = 0.043$ ). CES was found to be the only variable significantly associated with response (Supplementary Table S15), independently of HER2 status (Supplementary Tables S15–S16).

#### Edinburgh-based dataset: CES and endocrine sensitivity

We evaluated a dataset of 120 postmenopausal patients with HR<sup>+</sup> disease treated with letrozole for at least 12 weeks in the

**Table 2.** Clinical-pathologic characteristics and subtype distribution of the 4 testing sets

	MDACC N (%)	Malaga N (%)	Marsden N (%)	Edinburgh N (%)
Treatment	CT	CT	ET	ET
N	272	180	103	120
HER2 status <sup>a</sup>				
HER2-negative	272 (100%)	180 (100%)	89 (86%)	31 (69%)
HER2-positive	0 (0%)	0 (0%)	14 (14%)	14 (31%)
Age (mean)	50.1	50.0	53.7	76.1
Menopausal status				
Premenopausal	NA	108 (60%)	0 (0%)	0 (0%)
Postmenopausal	NA	72 (40%)	103 (100%)	120 (100%)
Tumor stage				
T0-T1	19 (7%)	18 (10%)	60 (58%)	10 (9%)
T2	142 (52%)	115 (67%)		42 (36%)
T3-T4	111 (41%)	39 (23%)	43 (42%)	63 (55%)
Node				
N0	96 (35%)	67 (37%)	61 (59%)	86 (72%)
N1	133 (49%)	61 (34%)	39 (38%)	34 (28%)
N2-N3	43 (16%)	52 (29%)	3 (3%)	
Grade				
G1	28 (11%)	27 (16%)	15 (15%)	13 (11%)
G2	136 (53%)	96 (57%)	63 (62%)	82 (68%)
G3	91 (36%)	46 (27%)	24 (23%)	25 (21%)
ET response rate <sup>b</sup>	NA	NA	53%	72%
CT response rate				
pCR breast/axilla	8.8%	6.7%	NA	NA
PAM50				
Luminal A	141 (52%)	54 (30%)	37 (36%)	—
Luminal B	102 (38%)	105 (58%)	20 (19%)	—
HER2-E	6 (2%)	7 (4%)	12 (12%)	—
Basal-like	7 (2%)	14 (8%)	4 (4%)	—
Normal-like	16 (6%)	—	30 (29%)	—

Abbreviations: CT, chemotherapy, ET, endocrine therapy.

<sup>a</sup>Edinburgh dataset has 75 patients without clinical HER2 status.

<sup>b</sup>The definition of ET response is different in the Marsden and Edinburgh data sets. Clinical tumor response (complete and partial response vs. stable and progressive disease) was used as the endpoint in the Marsden dataset. Response was evaluated by imaging ultrasound in the Edinburgh dataset. Clinical tumor response was defined as tumor volume shrinkage of at least 70% by 90 days of treatment.

neoadjuvant setting (Supplementary Fig. S2A). Two patients of 120 achieved a complete response. Similar to previous results, CES as a continuous variable was found to be the only variable significantly associated with a  $\geq 70\%$  reduction in tumor volume by 90 days (Supplementary Fig. S2B), even within HER2-negative disease (Supplementary Fig. S2C).

**Table 3.** CES association with chemotherapy sensitivity in the MDACC-based dataset

Signatures	N	pCR rate	Univariate analysis				Multivariable analysis			
			OR	Lower 95%	Upper 95%	P	OR	Lower 95%	Upper 95%	P
Age (cont. variable)	—	—	1.0	0.93	1.02	0.251	1.0	0.92	1.02	0.205
Tumor size										
T0-T2	153	8%	1.0	—	—	—	1.0	—	—	—
T3-T4	107	9%	1.1	0.47	2.63	0.813	0.6	0.22	1.70	0.341
Nodal status										
N0	96	7%	1.0	—	—	—	1.0	—	—	—
N1	125	9%	1.2	0.46	3.29	0.685	0.9	0.30	2.78	0.882
N2-3	39	13%	1.9	0.56	6.29	0.312	1.0	0.24	4.51	0.965
Grade										
1	26	4%	1.0	—	—	—	1.0	—	—	—
2	130	4%	1.0	0.11	8.93	1.000	0.7	0.07	6.88	0.753
3	89	17%	5.1	0.64	40.34	0.125	1.8	0.18	18.42	0.608
PAM50										
Luminal A	134	3%	1.0	—	—	—	1.0	—	—	—
Luminal B	99	15%	5.8	1.86	18.08	0.002	1.2	0.25	6.28	0.792
HER2-E	6	0%	0.0	—	—	0.989	0.0	—	—	0.991
Basal-like	7	29%	13.0	1.91	88.50	0.009	0.4	0.02	9.97	0.586
Normal-like	14	14%	5.4	0.90	32.69	0.065	1.7	0.23	12.75	0.602
CES										
CES-E	134	2%	1.0	—	—	—	—	—	—	—
CES-U	67	9%	4.3	1.04	17.75	0.044	—	—	—	—
CES-C	59	24%	13.6	3.73	49.46	<0.001	—	—	—	—
CES (cont. variable)	—	—	0.2	0.08	0.40	<0.001	0.2	0.03	0.77	0.022

**Table 4.** CES association with chemotherapy sensitivity in the Malaga dataset

Signatures	N	RCB0/1 rate	Univariate analysis				Multivariable analysis			
			OR	Lower 95%	Upper 95%	P	OR	Lower 95%	Upper 95%	P
Age (cont. variable)	—	—	1.0	0.95	1.02	0.331	1.0	0.96	1.07	0.599
Tumor size										
T0-T2	133	22%	1.0	—	—	—	1.0	—	—	—
T3-T4	39	10%	0.4	0.13	1.25	0.116	0.4	0.09	1.90	0.260
Grade										
1	27	7%	1.0	—	—	—	1.0	—	—	—
2	96	16%	2.3	0.50	10.82	0.286	1.6	0.26	9.31	0.625
3	46	35%	6.7	1.40	31.82	0.017	3.0	0.40	23.34	0.283
PAM50										
Luminal A	54	9%	1.0	—	—	—	1.0	—	—	—
Luminal B	105	20%	2.4	0.87	6.91	0.090	0.9	0.19	4.34	0.905
HER2-E	7	14%	1.6	0.16	16.43	0.677	0.1	0.00	3.19	0.188
Basal-like	14	50%	9.8	2.43	39.51	0.001	0.1	0.00	3.40	0.214
CES										
CES-E	83	10%	1.0	—	—	—	—	—	—	—
CES-U	29	17%	2.0	0.58	6.54	0.277	—	—	—	—
CES-C	68	31%	4.2	1.72	10.22	0.002	—	—	—	—
CES (cont. variable)	—	—	0.2	0.09	0.44	<0.001	0.2	0.07	0.76	0.016

**Prognosis, intrinsic subtype, and chemoendocrine sensitivity**

To better understand the relationship between prognosis, intrinsic biology, and chemoendocrine sensitivity, we pulled together PAM50 data from many different data sets for a total of 6,007 primary breast cancers representing all subtypes (Fig. 2). The results revealed that in the ROR-low group, 94.9% of cases were identified as CES-E and 100% were of the Luminal A subtype. In the ROR-high, 92.1% of the samples were identified as CES-C; non-luminal and Luminal B subtypes represented 64.3% and 35.7% of the ROR-high/CES-C cases, respectively.

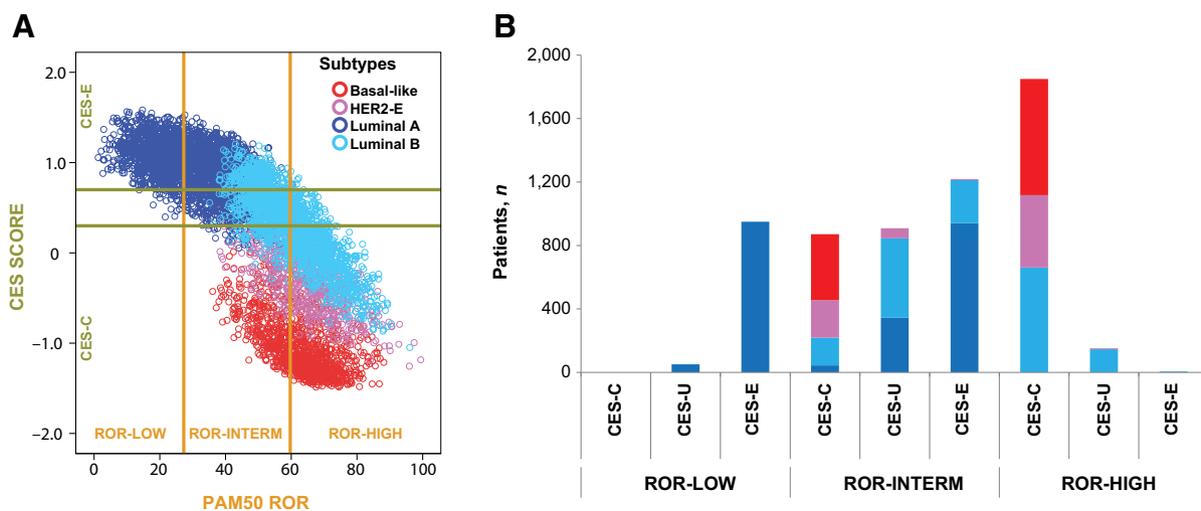
In the ROR-intermediate group, high heterogeneity was observed. In terms of intrinsic biology, Luminal A, Luminal B, and non-Luminal subtypes represented 44.4%, 31.5%, and 24.1%, respectively. In terms of chemo/endocrine-sensitivity, CES-E, CES-U, and CES-C represented 40.6%, 30.3%, and 29.1%, respectively. As expected, the vast majority of ROR-

intermediate/CES-E samples (77.3%) were of the Luminal A subtype.

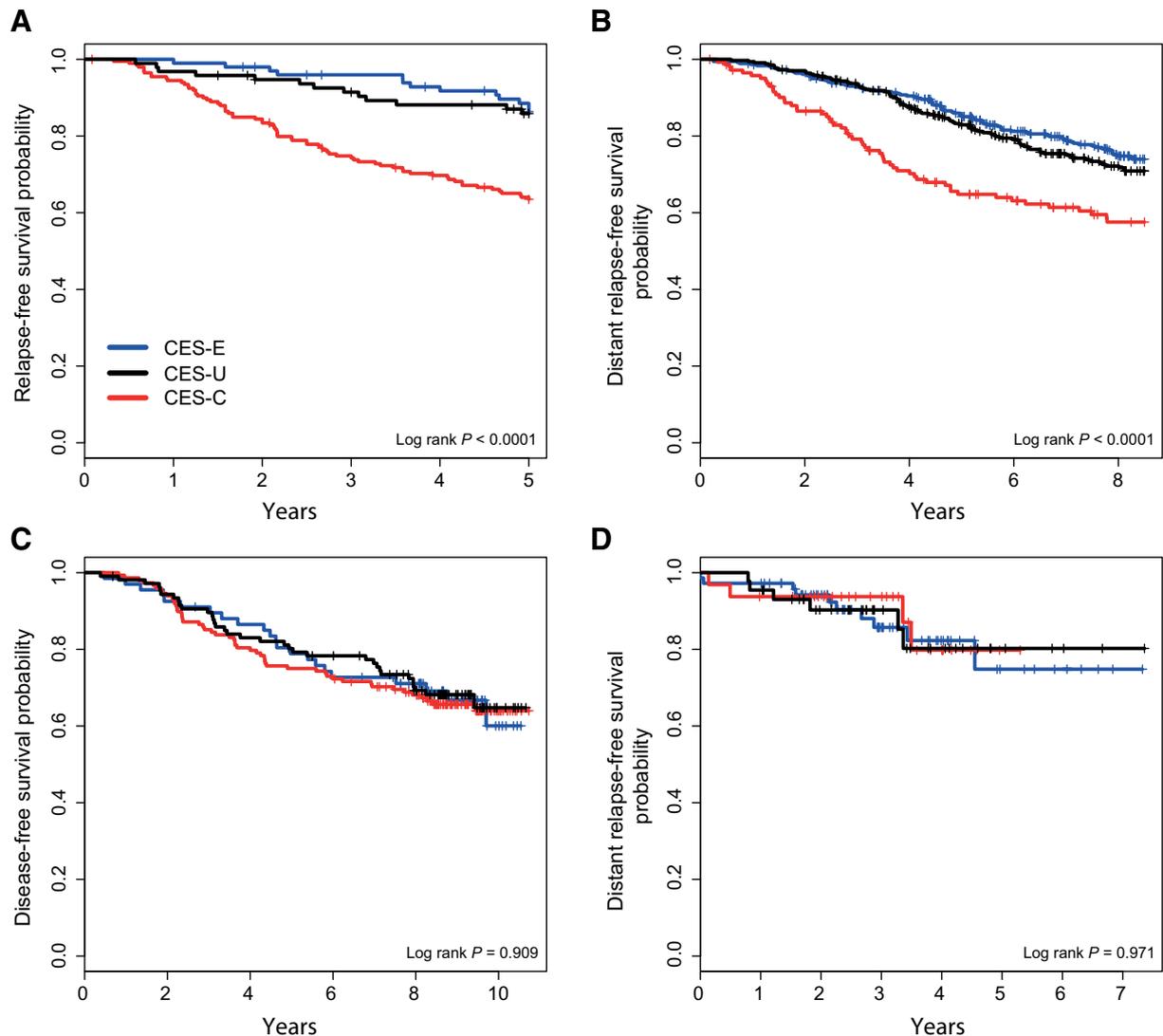
**Survival outcome of CES within HR<sup>+</sup>/ROR-intermediate disease**

To continue exploring the value of CES within HR<sup>+</sup>/ROR-intermediate disease, we evaluated the association of CES with survival outcome in HR<sup>+</sup>/ROR-intermediate early breast cancer in 4 independent data sets of patients treated with no adjuvant systemic therapy ( $n = 189$ ), adjuvant tamoxifen-only ( $n = 846$ ) or adjuvant chemotherapy and endocrine therapy ( $n = 322$  and  $n = 148$ ).

In patients with node-negative disease treated without adjuvant systemic therapy, CES (as a continuous variable or as group categories) was found significantly associated with distant relapse-free survival (Fig. 3A). The hazard ratio between the

**Figure 2.**

Prognosis (PAM50 ROR), intrinsic subtype, and CES in 6,007 primary breast cancers. **A**, A scatter plot of CES score and ROR score, colored by subtype, is shown. The two horizontal lines indicate the cutoffs of each CES group. The two vertical lines indicate the cutoffs of each PAM50 ROR group. **B**, Number of patients in each CES group based on ROR. Each bar is colored by subtype.

**Figure 3.**

Survival outcomes in ER<sup>+</sup> early breast cancer with ROR-intermediate disease. **A**, Node-negative disease treated without adjuvant systemic therapy. **B**, Node-negative and node-positive disease treated with adjuvant tamoxifen only. **C**, Node-positive disease treated with adjuvant chemotherapy and endocrine therapy in the GEICAM/9906 clinical trial. **D**, Node-negative and node-positive disease treated with neoadjuvant chemotherapy and adjuvant endocrine therapy.

CES-C group versus the CES-E group was 2.68 (95% confidence interval, 0.163–0.858). Similar results were obtained in the dataset where patients were treated with adjuvant tamoxifen-only (Fig. 3B). However, CES (as a continuous variable or as group categories) was not found significantly associated with survival outcome in 2 independent cohorts of patients treated with (neo) adjuvant chemotherapy and endocrine therapy (Fig. 3C and D).

## Discussion

Our results are the first to confirm, in a randomized setting, an inverse relationship of endocrine and chemotherapy sensitivity in ER<sup>+</sup> breast cancer. Previous evidence has suggested an inverse relationship of proliferation- and ER-related biological processes regarding endocrine and chemotherapy sensitivity of

ER<sup>+</sup> breast cancer. For example, two independent studies showed an inverse correlation between a 200-gene ER reporter score, or between TAU expression, an ER-related gene, and endocrine sensitivity and chemosensitivity (38, 39). In addition, high recurrence score measured by Oncotype DX (Genomic Health, Inc.) predicted little or no benefit from adjuvant tamoxifen therapy in the NSABP-B14 trial, but at the same time also predicted substantial benefit from adjuvant CMF chemotherapy in the NSABP-B20 trial (40, 41). These results fit with our results showing that virtually all patients with ROR-high disease are identified as CES-C; however, our data also highlight that within ROR-high/CES-C disease not all ER<sup>+</sup>/HER2<sup>-</sup> samples are luminal (i.e., Luminal A or B) since non-luminal disease (i.e., Basal-like and HER2-enriched) can also be identified. According to our results (Fig. 2), the chemotherapy benefit of

ROR-high/non-luminal tumors within HR<sup>+</sup>/HER2<sup>-</sup> disease is likely even greater than ROR-high/Luminal B tumors.

Our results also suggest that a main driver of endocrine therapy sensitivity and chemotherapy sensitivity within ER<sup>+</sup>/HER2<sup>-</sup> disease is the Basal-like versus Luminal A intrinsic biology. To capture both biological states in each individual sample, we calculated the correlation coefficients of each sample to both PAM50 centroids (i.e., Luminal A and Basal-like) and then subtracted both coefficients. Thus, instead of choosing a gene signature (e.g., a proliferation-based signature) of the many signatures that can discriminate between both subtypes in one way or another, we decided to incorporate into a score the Basal-like versus Luminal A intrinsic state of each tumor as identified by the PAM50 subtype predictor. Of note, the PAM50 genes were originally selected for their ability to capture the intrinsic biology displayed by 1,900 genes (i.e., the so-called intrinsic gene list). In fact, in the TCGA, intrinsic subtype defined by PAM50 captured the vast majority of the biological diversity displayed by most molecular data types analyzed (1).

From a clinical perspective, our results support current breast cancer guidelines for the systemic treatment of early HR<sup>+</sup>/HER2<sup>-</sup> breast cancer. On the one hand, patients with a low-ROR score and a low tumor burden (i.e., <10% risk of distant relapse at 10 years) are recommended to be treated with endocrine therapy only (42). Indeed, our results suggest that these patients have tumors that are highly endocrine sensitive and have low chemotherapy sensitivity. On the other hand, patients with high-risk HR<sup>+</sup>/HER2<sup>-</sup> disease are recommended to be treated with endocrine therapy and chemotherapy. According to our analysis, this group is the one with high chemotherapy benefit and low endocrine benefit. Regarding endocrine therapy in this group, the main issue is that we do not have survival data suggesting that CES-C tumors do not benefit at all from endocrine therapy. Therefore, withdrawal of a potentially efficacious treatment strategy such as endocrine therapy in a patient with an ER<sup>+</sup> tumor (as defined by the ASCO/CAP guidelines) that is identified as CES-C or ROR-high should not be recommended today, although in patients whose tumors contain low levels of ER (1% to 10%), ASCO/CAP recommend to discuss the pros and cons of endocrine therapy. A large randomized adjuvant trial involving thousands of patients to answer this particular question is unlikely to happen.

Although the clinical implications of CES in low- and high-risk HR<sup>+</sup>/HER2<sup>-</sup> disease are minimal, the observation that intermediate-risk HR<sup>+</sup>/HER2<sup>-</sup> disease, which represents ~30% of newly diagnosed breast cancer, is biologically heterogeneous with a range of chemotherapy sensitivities might have implications for the interpretation of two ongoing prospective clinical trials. In the TailorX phase III trial, 4,500 patients with HR<sup>+</sup>/HER2<sup>-</sup> node-negative early breast cancer with intermediate RS have been randomly assigned to adjuvant chemotherapy or no chemotherapy. According to our analysis, this intermediate group might be composed of at least 3 groups with different chemotherapy sensitivities. Of note, the CES-U group seems to be a genuine gray area where decisions regarding the need of chemotherapy might be difficult. A similar situation might occur in the RxPONDER phase III clinical trial where patients with HR<sup>+</sup>/HER2<sup>-</sup> early breast cancer, and 1-3-positive lymph nodes, with low/intermediate risk are being randomly assigned to adjuvant chemotherapy or not. A potential explanation is that OncotypeDX RS, as well as other prognostic gene expression-based tests, such as PAM50 ROR

or MammaPrint (43), have been specifically designed or trained to predict outcome and not intrinsic tumor biology or treatment sensitivity. Although a strong negative correlation is observed between ROR (risk) and CES (drug sensitivity), there are substantial differences between them at the individual level (~40% discordance).

There are several caveats to our study. First, this is a retrospective study involving heterogeneous patient populations and the results need to be confirmed in a prospective clinical trial(s). Second, although the data presented here validate CES from a clinical perspective, further analytical validation will be needed, since in most data sets, except the Malaga set, the research-based version of PAM50 was used. However, the fact that CES (as a continuous variable and the 2 cutoff points) predicted pCR in the Malaga set suggests that analytical validation of this biomarker is feasible. Third, we did not evaluate the association of CES with survival data from a randomized clinical trial of adjuvant chemotherapy versus no adjuvant chemotherapy, or adjuvant endocrine therapy versus no adjuvant endocrine therapy. Thus, the predictive value of these signatures was evaluated only in the neoadjuvant setting where different tumor response endpoints were evaluated, most of which have been associated with patient survival (18, 44). Fourth, some of the signatures evaluated in the MDACC-based dataset, such as OncotypeDX recurrence score or genomic grade index, were derived from microarray-based data and thus are not the commercially available versions. Fifth, we were not able to demonstrate a consistent association of CES with endocrine response in HR<sup>+</sup> disease after excluding the HER2-positive cases. In the Edinburgh dataset, HER2 status was not available for all patients. Although we derived an ERBB2 expression-based surrogate definition of HER2 status and showed that CES is independently associated with response, this was not prespecified and does not meet REMARK guidelines. In addition, the association of CES with endocrine response did not reach statistical significance ( $P = 0.09$ ) in patients with HR<sup>+</sup>/HER2<sup>-</sup> disease in the Marsden dataset. Finally, patients from each of the data sets received different anthracycline/taxane-based chemotherapy regimens, schedules, and doses, and thus the ability of the signatures to predict response to particular chemotherapeutics or treatment regimens could not be tested.

Another important consideration of our study is that we did not attempt to identify an optimal cutoff(s) for CES but rather focused on the association of the continuous expression of CES with each endpoint. The main reason is that different gene expression-based platforms and protocols were used in each cohort and, thus, standardization of a biomarker cutoff point would have been difficult to achieve and most likely unreliable. In any case, the fact that all four testing sets gave very similar associations, and were found independently of the platform/protocol used, argues in favor of a robust finding.

To conclude, CES is a single genomic signature capable of measuring chemoendocrine sensitivity in HR<sup>+</sup>/HER2<sup>-</sup> breast cancer beyond intrinsic subtype, other genomic signatures, and the standard pathology variables. CES could be of particular clinical value in patients with HR<sup>+</sup>/HER2<sup>-</sup> intermediate-risk disease where the benefit of adjuvant multiagent chemotherapy is unclear.

#### Disclosure of Potential Conflicts of Interest

A. Prat reports receiving commercial research grants from and is a consultant/advisory board member for Nanostring Technologies. A. Lluch is a consultant/

advisory board member for Novartis, Pfizer, and Roche. J.S. Parker is listed as a co-inventor on a pending patent on the PAM50, which is owned by University Genomics, LLC and licensed to Nanostring. M. Dowsett reports receiving speakers bureau honoraria from Myriad, and is a consultant/advisory board member for Genoptix and Nanostring. C.M. Perou is an employee of, holds ownership interest (including patents) in, and is a consultant/advisory board member for Bioclassifier LLC. No potential conflicts of interest were disclosed by the other authors.

## Disclaimer

The funders did not have any role in the study design, conduct, or decision to submit the manuscript for publication.

## Authors' Contributions

**Conception and design:** A. Prat, J. Albanell, P. Sánchez-Rovira, B. Adamo, C.M. Perou, E. Alba

**Development of methodology:** A. Prat, P. Sánchez-Rovira, B. Jimenez, M. Martín, C.M. Perou

**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** A. Plazaola, N. Ribelles, B. Jimenez, M. Dowsett, M. Martín, J.M. Dixon, C.M. Perou, E. Alba

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** A. Prat, L. Calvo, J.I. Chacón, P. Sánchez-Rovira, M. Muñoz, L. Paré, J.S. Parker, A.A. Bin Aiderus, B. Adamo, E. Carrasco, M. Martín, C.M. Perou, E. Alba

**Writing, review, and/or revision of the manuscript:** A. Prat, A. Lluch, A.K. Dunbier, L. Calvo, J. Albanell, J. de la Haba-Rodríguez, A. Arcusa, J.I. Chacón, P. Sánchez-Rovira, M. Muñoz, J.S. Parker, B. Jimenez, R. Caballero, B. Adamo, M. Dowsett, E. Carrasco, M. Martín, J.M. Dixon, C.M. Perou, E. Alba

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** A.K. Turnbull, P. Sánchez-Rovira, B. Jimenez, R. Caballero

**Study supervision:** P. Sánchez-Rovira, E. Alba

## Acknowledgments

This work was supported by funds from the NCI Breast SPORE program (P50-CA58223-09A1), by RO1-CA148761 (C.M. Perou), by Instituto de Salud Carlos III - PI13/01718 (A. Prat), by a Career Catalyst Grant from the Susan Komen Foundation (A. Prat), by Breast Cancer Now (formerly Breakthrough Breast Cancer), by the Royal Marsden NIHR Biomedical Research Centre, by Banco Bilbao Vizcaya Argentaria (BBVA) Foundation (A. Prat), and by the Breast Cancer Research Foundation. This work was also supported by funds from FEDER (RETICC): RD12/0036/0076 (J. Albanell), RD12/0036/0051 (J. Albanell), RD12/0036/0070 (A. Lluch), and RD12/0036/0076 (M. Muñoz).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 20, 2016; revised October 22, 2016; accepted November 7, 2016; published OnlineFirst November 30, 2016.

## References

- TCGA. Comprehensive molecular portraits of human breast tumours. *Nature* 2012;490:61–70.
- Prat A, Cheang MCU, Martín M, Parker JS, Carrasco E, Caballero R, et al. Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. *J Clin Oncol* 2013;31:203–9.
- Ades F, Zardavas D, Bozovic-Spasojevic I, Pugliano L, Fumagalli D, de Azambuja E, et al. Luminal B breast cancer: molecular characterization, clinical management, and future perspectives. *J Clin Oncol* 2014;32:2794–803.
- Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol* 2011;5:5–23.
- Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. *Nat Rev Clin Oncol* 2012;9:48–57.
- Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
- Martín M, Prat A, Rodríguez-Lescure Á, Caballero R, Ebbert MW, Munárriz B, et al. PAM50 proliferation score as a predictor of weekly paclitaxel benefit in breast cancer. *Breast Cancer Res Treat* 2013;138:457–66.
- Prat A, Carey LA, Adamo B, Vidal M, Taberero J, Cortés J, et al. Molecular features and survival outcomes of the intrinsic subtypes within HER2-positive breast cancer. *J Natl Cancer Inst* 2014;106:pii:dju152. doi: 10.1093/jnci/dju152.
- Usary J, Zhao W, Darr D, Roberts PJ, Liu M, Balletta L, et al. Predicting drug responsiveness in human cancers using genetically engineered mice. *Clin Cancer Res* 2013;19:4889–99.
- von Minckwitz G, Untch M, Blohmer J-U, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol* 2012;30:1796–804.
- Prat A, Parker JS, Fan C, Perou CM. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. *Breast Cancer Res Treat* 2012;135:301–306.
- Prat A, Fan C, Fernández A, Hoadley KA, Martinello R, Vidal M, et al. Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy. *BMC Med* 2015;13:1–11.
- Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, Prat A, et al. Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z1031. *J Clin Oncol* 2011;29:2342–9.
- Dunbier AK, Anderson H, Ghazoui Z, Salter J, Parker JS, Perou CM, et al. Association between breast cancer subtypes and response to neoadjuvant anastrozole. *Steroids* 2011;76:736–40.
- Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer 2013. *Ann Oncol* 2013.
- Alba E, Calvo L, Albanell J, De la Haba JR, Arcusa Lanza A, Chacon JJ, et al. Chemotherapy (CT) and hormonotherapy (HT) as neoadjuvant treatment in luminal breast cancer patients: results from the GEICAM/2006-03, a multicenter, randomized, phase-II study. *Ann Oncol* 2012;23:3069–74.
- Wolff AC, Hammond MEH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2006;25:118–45.
- Ogston KN, Miller ID, Payne S, Hutcheon AW, Sarkar TK, Smith I, et al. A new histological grading system to assess response of breast cancers to primary chemotherapy: prognostic significance and survival. *Breast* 2003;12:320–7.
- Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nat Biotech* 2008;26:317–25.
- Hatzis C, Pusztai L, Valero V, Booser DJ, Esserman L, Lluch A, et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. *JAMA* 2011;305:1873–81.
- Prat A, Galván P, Jimenez B, Buckingham W, Jeiranian HA, Schaper C, et al. Prediction of response to neoadjuvant chemotherapy using core needle biopsy samples with the Prosigna assay. *Clin Cancer Res* 2016;22:560–6.
- Dunbier AK, Anderson H, Ghazoui Z, Folklerd EJ, A'Hern R, Crowder RJ, et al. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. *J Clin Oncol* 2010;28:1161–7.
- Smith IE, Walsh G, Skene A, Lombardi A, Mayordomo JJ, Detre S, et al. A Phase II placebo-controlled trial of neoadjuvant anastrozole alone or with gefitinib in early breast cancer. *J Clin Oncol* 2007;25:3816–22.
- Turnbull AK, Arthur LM, Renshaw L, Larionov AA, Kay C, Dunbier AK, et al. Accurate prediction and validation of response to endocrine therapy in breast cancer. *J Clin Oncol* 2015;33:2270–8.

25. Fan C, Prat A, Parker JS, Liu Y, Carey LA, Troester MA, et al. Building prognostic models for breast cancer patients using clinical variables and hundreds of gene expression signatures. *BMC Med Genomics* 2011;4: 1–15.
26. Prat A, Parker JS, Fan C, Cheang MCU, Miller LD, Bergh J, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. *Ann Oncol* 2012;23: 2866–73.
27. Parker JS, Mullins M, Cheang MCU, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;27:1160–7.
28. Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res* 2010;16:5222–32.
29. Prat A, Lluch A, Albanell J, Barry WT, Fan C, Chacon JJ, et al. Predicting response and survival in chemotherapy-treated triple-negative breast cancer. *Br J Cancer* 2014;111:1532–41.
30. Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. *Nat Rev Clin Oncol* 2011;9:48–57.
31. Curtis C, Shah SP, Chin S-F, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature* 2012;486:346–52.
32. Horak CE, Pusztai L, Xing G, Trifan OC, Saura C, Tseng L-M, et al. Biomarker analysis of neoadjuvant doxorubicin/cyclophosphamide followed by ixabepilone or paclitaxel in early-stage breast cancer. *Clin Cancer Res* 2013; 19:1587–95.
33. Fan C, Prat A, Parker J, Liu Y, Carey L, Troester M, et al. Building prognostic models for breast cancer patients using clinical variables and hundreds of gene expression signatures. *BMC Med Genomics* 2011;4:3.
34. Dennis G, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, et al. DAVID: database for annotation, visualization, and Integrated discovery. *Genome Biol* 2003;4:R60.
35. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci U S A* 2001;98:5116–21.
36. Fan C, Oh DS, Wessels L, Weigelt B, Nuyten DSA, Nobel AB, et al. Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med* 2006;355:560–9.
37. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
38. Symmans WF, Hatzis C, Sotiriou C, Andre F, Peintinger F, Regitnig P, et al. Genomic index of sensitivity to endocrine therapy for breast cancer. *J Clin Oncol* 2010;28:4111–9.
39. Andre F, Hatzis C, Anderson K, Sotiriou C, Mazouni C, Mejia J, et al. Microtubule-associated protein-tau is a bifunctional predictor of endocrine sensitivity and chemotherapy resistance in estrogen receptor-positive breast cancer. *Clin Cancer Res* 2007;13:2061–7.
40. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
41. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006;24:3726–34.
42. Harris LN, Ismaila N, McShane LM, Andre F, Collyar DE, Gonzalez-Angulo AM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016;34:1134–50.
43. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AAM, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530–6.
44. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. *Lancet* 2014;384:164–72.

# Clinical Cancer Research

## A PAM50-Based Chemoendocrine Score for Hormone Receptor–Positive Breast Cancer with an Intermediate Risk of Relapse

Aleix Prat, Ana Lluch, Arran K. Turnbull, et al.

*Clin Cancer Res* Published OnlineFirst November 30, 2016.

**Updated version** Access the most recent version of this article at:  
doi:[10.1158/1078-0432.CCR-16-2092](https://doi.org/10.1158/1078-0432.CCR-16-2092)

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, contact the AACR Publications Department at [permissions@aacr.org](mailto:permissions@aacr.org).