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ORIGINAL REPORT

Genomic Index of Sensitivity to Endocrine Therapy for Breast Cancer

W. Fraser Symmans, Christos Hatzis, Christos Sotiriou, Fabrice Andre, Florentia Peintinger, Peter Regitnig, Guenter Daxenbichler, Christine Desmedt, Julien Domont, Christian Marth, Suzette Delaloge, Thomas Bauernhofer, Vicente Valero, Daniel J. Booser, Gabriel N. Hortobagyi, and Lajos Pusztai

See accompanying editorial on page 4101

A B S T R A C T

Purpose

We hypothesize that measurement of gene expression related to estrogen receptor α (ER; gene name *ESR1*) within a breast cancer sample represents intrinsic tumoral sensitivity to adjuvant endocrine therapy.

Methods

A genomic index for sensitivity to endocrine therapy (SET) index was defined from genes coexpressed with *ESR1* in 437 microarray profiles from newly diagnosed breast cancer, unrelated to treatment or outcome. The association of SET index and *ESR1* levels with distant relapse risk was evaluated from microarrays of ER-positive breast cancer in two cohorts who received 5 years of tamoxifen alone as adjuvant endocrine therapy (n = 225 and 298, respectively), a cohort who received neoadjuvant chemotherapy followed by tamoxifen and/or aromatase inhibition (n = 122), and two cohorts who received no adjuvant systemic therapy (n = 208 and 133, respectively).

Results

The SET index (165 genes) was significantly associated with distant relapse or death risk in both tamoxifen-treated cohorts (hazard ratio [HR] = 0.70, 95% CI, 0.56 to 0.88, $P = .002$; and HR = 0.76, 95% CI, 0.63 to 0.93, $P = .007$) and in the chemo-endocrine–treated cohort (HR = 0.19; 95% CI, 0.05 to 0.69, $P = .011$) independently from pathologic response to chemotherapy, but was not prognostic in two untreated cohorts. No distant relapse or death was observed after tamoxifen alone if node-negative and high SET or after chemo-endocrine therapy if intermediate or high SET.

Conclusion

The SET index of ER-related transcription predicted survival benefit from adjuvant endocrine therapy, not inherent prognosis. Prior chemotherapy seemed to enhance the efficacy of adjuvant endocrine therapy related to SET index.

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From The University of Texas M. D. Anderson Cancer Center, Houston TX; Nuvera Biosciences, Woburn, MA; Institut Jules Bordet, Brussels, Belgium; Institut Gustave Roussy, Villejuif, France; General Hospital Leoben, Loeben; Medical University of Graz, Graz; and Medical University of Innsbruck, Austria.

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Both W.F.S. and C.H. contributed equally to this work.

Data sets for the factorial study and tamoxifen-treated validation cohort are accessible via the GEO repository (http://www.ncbi.nlm.nih.gov/geo/) under accession IDs GSE17700 and GSE17705. Other datasets are linked to their original published reports.

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Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: W. Fraser Symmans, MD, Department of Pathology, Box 85, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030; e-mail: fsymmans@mdanderson.org.

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INTRODUCTION

Current challenges for adjuvant (postoperative) treatment of patients with hormone receptor–positive breast cancer include the ability to predict benefit from endocrine therapy independently from the natural history after appropriate locoregional treatment (prognosis), to predict sequential synergy from chemotherapy followed by endocrine therapy, and to optimize the selection, duration and sequence of endocrine treatments.[1-4] These challenges are each conceptually related to the function of estrogen receptor α (ER; gene name *ESR1*) in a patient's breast cancer. ER activates transcription of numerous genes, directly by binding to estrogen response elements within the promoter regions of some gene and secondarily through transcription initiated by ER-dependent transcription factors and cross-talk between ER at the cell membrane and tyrosine kinase signaling pathways.[5-11] Consequently, ER status is a principal determinant of overall gene transcription in breast cancers.[6,12,13] We hypothesized that measurement of the level of ER-associated gene expression in a patient's tumor sample would represent the extent of ER transcriptional activity and thus its likely dependence on estrogen stimulation and consequently would predict the intrinsic sensitivity of that tumor to endocrine therapy. We proceeded to identify an ER-related transcriptional signature and derived an index from

a large representative breast tumor cohort (unrelated to treatment or outcome), tested the relationship between this index and distant relapse risk in a different sample cohort from tamoxifen-treated patients, developed cutoff points to define three endocrine sensitivity classes, and then tested the predictive performance of these classes in a second independent sample cohort from tamoxifen-treated patients, in two sample cohorts from patients who did not receive any adjuvant systemic therapy, and in a cohort of patients who received neoadjuvant chemotherapy followed by adjuvant endocrine therapy (tamoxifen and/or aromatase inhibition).

PATIENTS AND METHODS

Patients and Samples

This research was conducted with approval and waiver of consent from the Institutional Research Board (protocol LAB04-0093). Patient and sample characteristics are summarized in Tables 1 and 2, respectively.

A discovery cohort was evaluated to identify genes with expression that is strongly related to *ESR1* expression and to describe an index to measure their combined expression, termed the sensitivity to endocrine therapy (SET) index. This cohort consisted of 437 available Affymetrix U133A microarray profiles from patients at The University of Texas M. D. Anderson Cancer Center (M. D. Anderson) who participated in a research protocol to obtain fine-needle aspiration (FNA) of newly diagnosed invasive breast cancer of American Joint Committee on Cancer stage I to III breast cancer (52% ER positive, 22% HER2 positive).[19,20]

A first validation cohort was studied to test the concept that the SET index would be related to distant relapse-free survival (DRFS) after adjuvant systemic treatment with tamoxifen alone for 5 years and to then identify thresholds that define categories of the SET index (low, intermediate, or high SET). This consisted of frozen tumor tissue from 245 patients with ER-positive invasive breast cancer that were profiled at Institut Jules Bordet (JBI) using Affymetrix U133A or U133Plus2.0 gene expression microarrays (Table 1).[14,15] We also evaluated potential inter-platform (U133A *v* U133Plus2.0) and inter-laboratory (JBI *v* M. D. Anderson) effects on SET index values using a 2 × 2 factorial study design in which residual cRNA from 17 cancers (representing the spectrum of SET index values) was profiled on both microarray platforms in both laboratories.

A second validation cohort was studied to independently assess the relationship between the predefined categories of the SET index and DRFS after adjuvant systemic treatment with tamoxifen alone for 5 years. This cohort consisted of frozen tumor tissue from 310 patients with ER-positive invasive breast cancer that were profiled at M. D. Anderson (n = 201) or JBI (n = 109) using only Affymetrix U133A gene expression microarrays.

Two different untreated cohorts were also studied to determine whether SET index represents the natural history of ER-positive breast cancer in patients who did not receive any systemic therapy. These consisted of gene expression data from Affymetrix U133A microarrays derived from frozen tumor samples from patients with node-negative, ER-positive

Table 1. Population Characteristics of the Validation Cohorts

	First Validation Cohort (Tamoxifen)								Second Validation Cohort (Tamoxifen)								Untreated Cohorts				Chemo/Endocrine (T/FAC, Tamoxifen/AI)	
	GUY		GUY2		KI		Total		AUS		IGR		OXF		Total		VDX		TRANS		MDA	
Characteristic	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
No. of patients	87		77		81		245		102		99		109		310		209		134		122	
Platform	Plus2		Plus2		U133A		U133A/Plus2		U133A		U133A		U133A		U133A		U133A		U133A		U133A	
Age, years																						
≤ 50	3	3	6	8	1	1	10	4	13	13	3	3	15	14	31	10	90	43	95	71	61	50
> 50	84	97	71	92	72	89	227	93	89	87	96	97	94	86	279	90	119	57	39	29	61	50
Mean	63		64		66		64		63		66		64		64		54		47		52	
SD	9		9		10		9		11		8		10		10		12		7		10	
Nodal status																						
Positive	58	67	36	47	48	59	142	58	46	45	35	35	37	34	118	38	0		0		80	66
Negative	29	33	41	53	22	27	92	38	51	50	64	65	66	61	181	58	209	100	134	100	42	34
NA	—	—	—	—	11	14	11	5	5	3	—	—	6	5	11	4	—	—	—	—	—	—
T stage																						
1	43	49	34	44	20	25	97	40	44	43	43	43	46	42	133	43	111	53	76	57	9	7
2	42	48	42	55	53	65	137	56	45	44	52	53	54	50	151	49	92	44	58	43	75	61
3	2	2	1	1	—	—	3	1	13	13	4	4	7	6	24	8	6	3	0		20	16
NA	—	—	—	—	8	10	8	3	—	—	—	—	2	2	2	1	—	—	—	—	—	—
Grade																						
1	17	20	14	18	12	15	43	18	21	21	24	24	21	19	66	21	4	2	29	22	12	10
2	48	55	34	44	42	52	124	51	59	58	52	53	51	47	162	52	36	17	69	51	75	61
3	16	18	24	31	14	17	54	22	20	20	23	23	17	16	60	19	102	49	36	27	35	29
NA	6	7	5	7	13	16	24	10	2	1	—	—	20	18	22	7	67	32	—	—	—	—
AJCC stage																						
I	17	20	22	29	6	7	45	18	24	24	32	32	32	29	88	28	111	53	76	57	1	1
II	68	78	54	70	64	79	186	76	63	62	57	58	63	58	183	59	92	44	58	43	78	64
III	2	2	1	1	0		3	1	6	6	10	10	6	6	22	7	6	3	0		43	35
NA	—	—	—	—	11	14	11	5	9	8	—	—	8	7	17	5	—	—	—	—	—	—
PR status																						
Positive	64	74	59	77	71	88	194	79	—	—	77	78	—	—	77	25	—	—	—	—	87	71
Negative	21	24	18	23	8	10	47	19	—	—	22	22	—	—	22	7	—	—	—	—	35	29
NA	2	2	—	—	2	2	4	2	102	100	—	—	109	100	211	68	209	100	134	100	—	—

Abbreviations: T/FAC, paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide; AI, aromatase inhibitor; GUY, Guys Hospital; KI, Karolinska Institute; AUS, Austria; IGR, Institut Gustave Roussy; OXF, Oxford; VDX, Veridex-Rotterdam; TRANS, TransBIG; MDA, M. D. Anderson Cancer Center; SD, standard deviation; NA, not available; AJCC, American Joint Committee on Cancer.

Table 2. Summary of Available Samples and the Total Number of Microarrays Analyzed

Factor	Sample Cohorts Evaluated					
	Discovery	First, Tamoxifen	Second, Tamoxifen	First, Untreated	Second, Untreated	Chemo-Endocrine
Dates samples collected	2000-2007	1987-1997	1978-2002	1980-1995	1980-1998	2000-2006
Insufficient RNA amount or quality	80	~60[14,15]	1	97[16]	104[17]	
Microarrays evaluated	460	245	309	286	198	
Microarrays failed	23	4	7	0	2	1*
ER-negative cases	NA	9	0	77	63	
DRFS unavailable or < 6 months	NA	7	4	1	0	9*
Total microarrays analyzed	437	225	298	208	133	122*[18]

Abbreviations: ER, estrogen receptor; DRFS, distant relapse-free survival.
*A published subset of our discovery cohort, from whom we excluded one microarray that failed our quality control, and nine patients who had only received endocrine therapy as palliative treatment (n = 7), refused adjuvant endocrine therapy (n = 1), or were lost to follow-up (n = 1).[18]

breast cancer that were profiled at Veridex (Raritan, NJ; n = 209)[16] or JBI (n = 134; Table 1).[14,17]

We also studied a chemo-endocrine cohort of 131 patients with ER-positive breast cancer and acceptable microarray quality (subset of the discovery cohort) who received uniform neoadjuvant chemotherapy with paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide (T/FAC), of whom 122 (Table 1) subsequently received adjuvant endocrine therapy with tamoxifen (n = 40), an aromatase inhibitor (n = 53), or both in sequence (n = 29).[18]

Breast cancers were defined as ER positive if nuclear immunostaining was ≥ 10% tumor cells or Allred score was ≥ 3,[21] or if enzyme immunoassay identified more than 10 femtomoles of ER/mg protein. The details of our methods for RNA purification and microarray hybridization have been reported previously.[14,15,17-20] Briefly, a single-round T7 amplification was used to generate biotin-labeled cRNA for hybridization to oligonucleotide microarrays (U133A GeneChip; Affymetrix, Santa Clara, CA). Raw intensity files (.CEL) from each microarray were processed using MAS5.0 (R/Bioconductor, www.bioconductor.org) to generate probe-level intensities and normalized to a median array intensity of 600, transformed to log2 values, and scaled by the expression levels of 1,322 breast cancer reference genes within each sample normalized to median values in a reference cohort.

Identification of ER-Related Genes

ER reporter genes were identified by their coexpression with ER gene (*ESR1*, probe set 205225_at) based on the Spearman's rank correlation coefficient in the discovery cohort (n = 437).[22] The size of the ER gene signature was determined, accounting for sampling variability by bootstrap resampling, and pruned to remove probe sets that contained cross-hybridizing probes, mapped to multiple genomic locations, were strongly associated with proliferation, or exhibited significant stromal bias in a set of matched FNAs and core biopsy samples of breast cancer from 38 different patients. The final signature included 106 genes with positive and 59 genes with negative correlation with *ESR1* (Data Supplement).

Statistical Analysis

DRFS was defined as the interval from breast surgery until diagnosis of distant metastasis or death from any cause.[23] The dependence of the hazard rate of distant relapse on the continuous SET index was modeled by a smoothing spline approximation with 2 *df*. The 10-year DRFS was estimated through a Cox proportional hazards model using the spline approximation of the SET index as the only covariate. The baseline cumulative hazard rate was estimated from the Cox model based on the Nelson-Aalen estimator, and the predicted rate of distant relapse was then obtained from the Breslow-type estimator of the survival function. CIs of the survival estimate were calculated based on the Tsiatis variance estimates of the cumulative log hazards.[24] Pathologic response to neoadjuvant chemotherapy was defined by the residual cancer burden (RCB).[25]

SET index values were classified as low, intermediate, or high based on cutoff values determined from the first validation cohort by fitting a Cox model of the trichotomous SET variable versus DRFS using different thresholds. Nontrivial thresholds that jointly maximized the log-profile likelihood surface for this model were selected as most informative cut points for predicting DRFS.[26] The same thresholds were maintained for subsequent validation analyses. The independent prognostic value of the SET index was assessed in multivariate Cox regression analyses based on the likelihood ratio test. All statistical computations were performed in R (v. 2.8.1, R Development Core Team, Vienna, Austria, 2008).

RESULTS

Definition of SET Index

Details of the individual ER-related genes, components of the index, and reproducibility of the genomic measurements are presented in the Data Supplement. We developed an index of *ESR1*-associated transcription from the mean expression levels X_P and X_N of 106 positively and 59 negatively correlated signature genes in a given sample. An endocrine index, defined as $EI = X_N + 0.5 (X_P - X_N)$, is higher in ER-positive tumors because the mean expression level of the positively correlated genes (X_P) is greater than that of the negatively correlated genes (X_N). This can be simplified to $EI = 0.5 (X_N + X_P)$. The EI was further scaled, first linearly to the range of 0 to 10, then through unconditional Box-Cox power transformation to normalize its distribution. The genomic index of sensitivity to endocrine therapy was then calculated as $SET = \max [0, 10 (EI - 9.48)^{1.24}]$. SET index values from samples hybridized on U133Plus2 arrays were adjusted for platform effects before further analysis due to bias observed between different Affymetrix microarrays (Data Supplement).

Relationship With Distant Relapse After Adjuvant Tamoxifen Therapy

In the first validation cohort, we observed a significant association between the SET index (continuous) and the risk (hazard rate) for distant relapse or death ($P = .003$, Fig 1A), but no significant relationship for *ESR1* expression (Fig 1B). The marginal (unadjusted) hazard ratio (HR) for the continuous SET index (as a linear term) was 0.70 (95% CI, 0.56 to 0.88; $P = .002$). The continuous SET index (HR = 0.65; 95% CI, 0.46 to 0.91; $P = .013$) and tumor size (T2-3 *v* T1; HR = 2.32; 95% CI, 1.03 to 5.24; $P = .041$) were independently predictive of DRFS after adjuvant tamoxifen treatment in multivariate Cox analysis adjusted for *ESR1*, age, nodal status, and grade (Data Supplement).

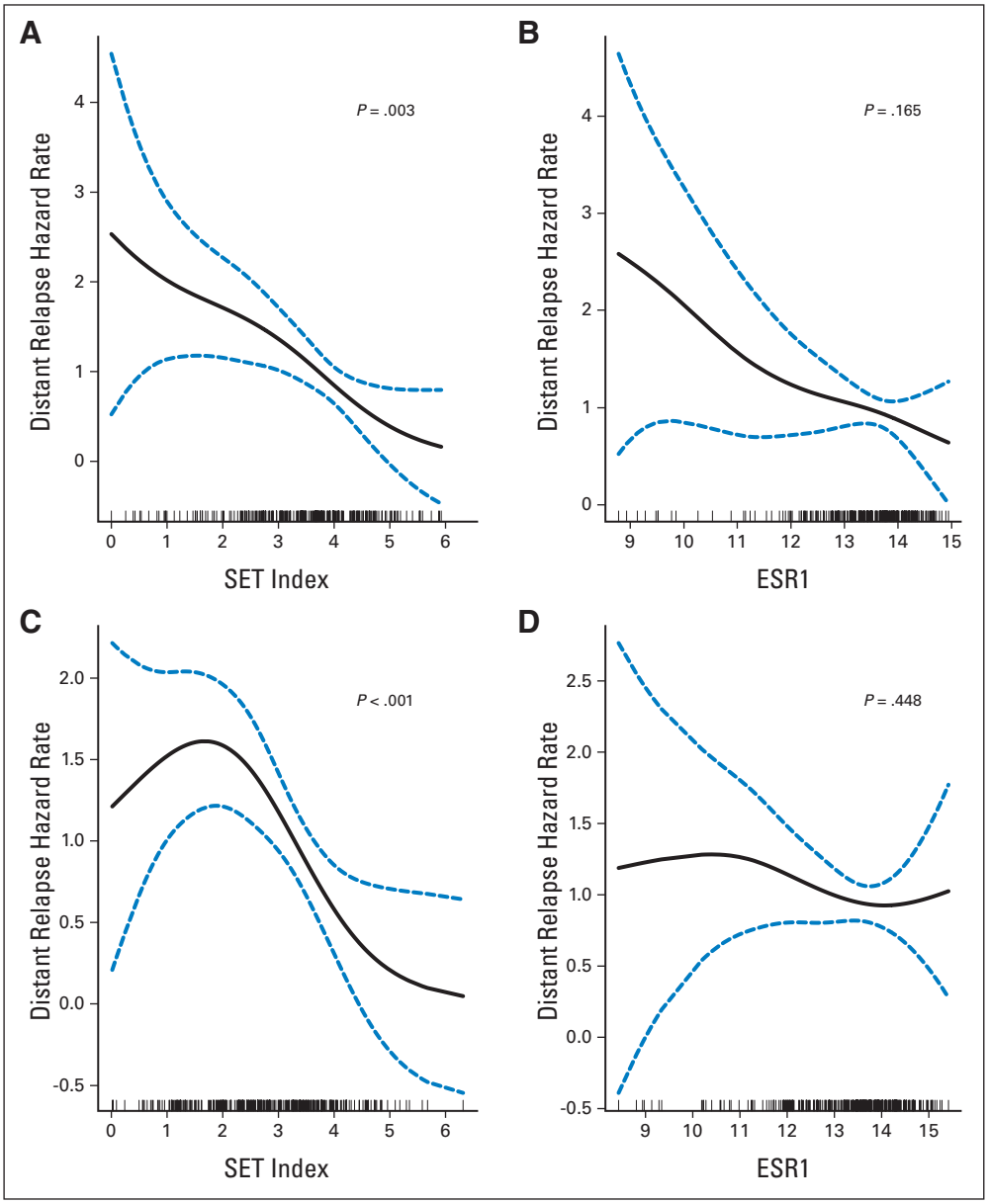

Fig 1. Hazard rate for distant relapse or death in 225 estrogen receptor (ER) – positive tamoxifen-treated patients from first validation cohort as a function of the genomic sensitivity to endocrine therapy (SET) index (A) and log2-transformed *ESR1* expression (B). (C, D) Corresponding profiles for the 298 ER-positive tamoxifen-treated patients from the second validation cohort. The dashed lines show the 95% pointwise CIs of the hazard rates. A risk of 1.0 corresponds to lack of covariate effect. *P* values are from the likelihood ratio test.

Three Classes of Endocrine Sensitivity Defined by SET Index

Having validated the concept that higher SET index (as a continuous measure of ER-related transcription) is associated with improved DRFS after adjuvant tamoxifen, we sought to establish clinically useful categories. Two cut points (corresponding to index values 2.68 and 3.66) were chosen to maximize the association of the trichotomous SET index with distant relapse events or death that occurred within the first 8 years of follow-up (Fig 2A).

Second Validation of Association With DRFS After Adjuvant Tamoxifen Therapy

A significant association between the SET index (continuous) and the hazard rate for distant relapse or death within 10 years was confirmed in the second independent validation cohort of samples from tamoxifen-treated patients ($P < .001$; Fig 1C), and again there was no significant relationship for *ESR1* expression (Fig 1D). The marginal (unadjusted) HR for DRFS of the continuous SET index (as a linear term) was 0.76 (95% CI, 0.63 to 0.93; $P = .007$). Furthermore, the previously defined category of high SET index in 24% of this cohort was associated with significantly improved DRFS, compared with intermediate or low SET categories (HR = 0.25, 95% CI, 0.10 to 0.63, $P < .001$; Fig 2B). The point estimates of DRFS for high, intermediate, and low SET index categories in this independent validation cohort at 5 years of follow-up were 94.1% (95% CI, 88.7% to 99.9%), 87.5% (95% CI, 80.5% to 95.1%), and 79.4% (95% CI, 72.9% to 86.5%), respectively, and point estimates at 10 years of follow-up were 92.1% (95% CI, 85.6% to 99.1%), 73.6% (95% CI, 63.5% to 85.3%), and 66.8% (95% CI, 58.8% to 76%), respectively.

Of note, the 10-year point estimate of DRFS in the high SET group was 100% (95% CI, 100% to 100%; no events) in patients with node-negative disease (Fig 2C) and 80% (95% CI, 65.5% to 97.4%) in

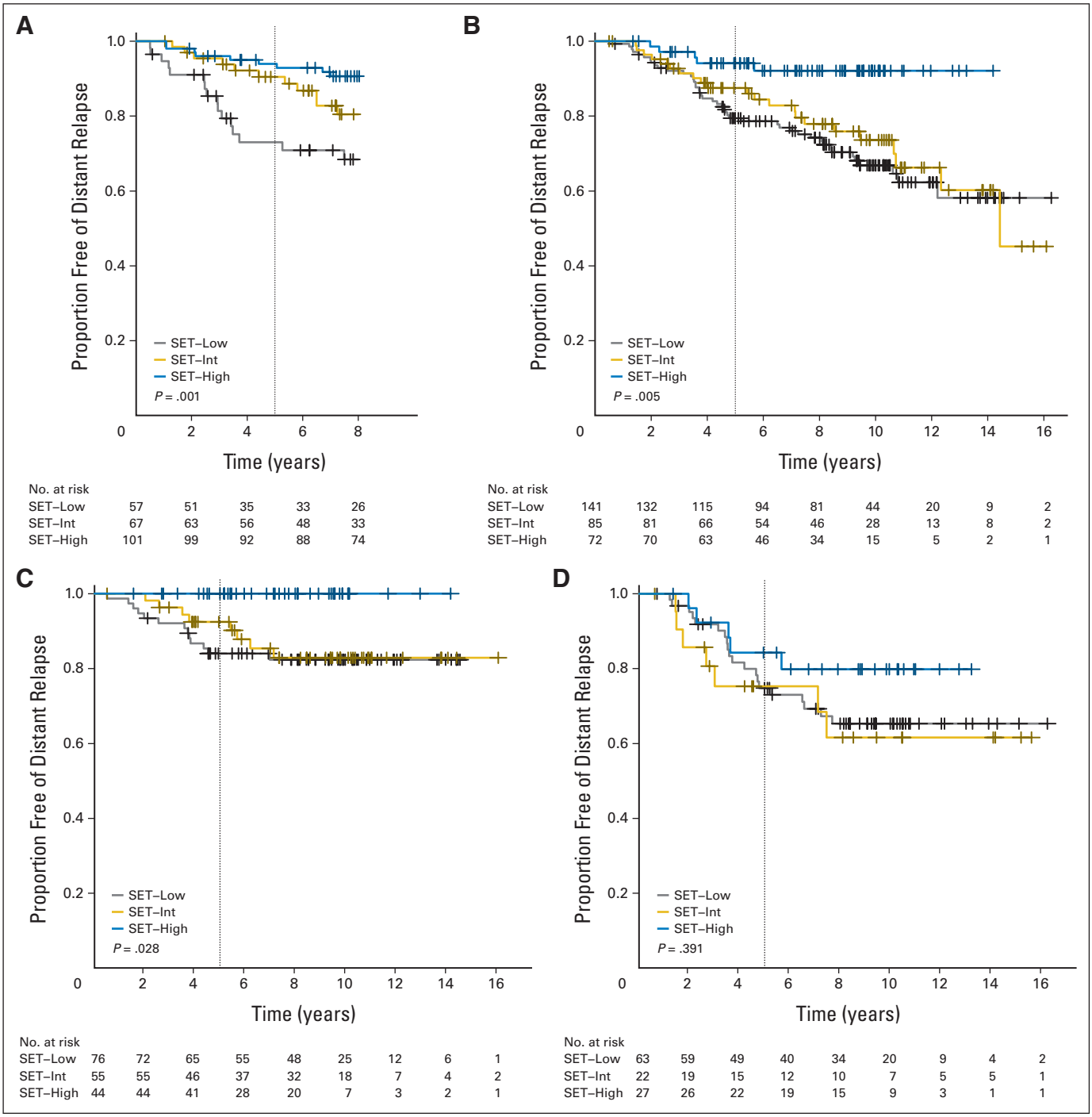

Fig 2. Kaplan-Meier estimates of relapse-free survival in patients treated with adjuvant tamoxifen in (A) the first validation cohort, with follow-up censored at 8 years, to define the thresholds for sensitivity to endocrine therapy (SET) index categories, and (B) the second independent validation cohort presented in toto with complete follow-up, and presented separately for the subsets with (C) node-negative and (D) node-positive breast cancer. Endocrine sensitivity groups were defined by the SET index. *P* values are from the log-rank test.

patients with node-positive disease (Fig 2D). High SET index and node-negative status were independently predictive of DRFS in a multivariate Cox model that included age, tumor size, grade, and Allred score for ER (Table 3).

Prognosis Without Adjuvant Systemic Therapy

Neither the gene expression level of ER (*ESR1*) nor the SET index were associated with the 5-year DRFS in two different cohorts of patients with ER-positive, node-negative breast cancer who did not receive any adjuvant systemic therapy (Figs 3A and 3B).

Association With DRFS After Adjuvant Chemo-Endocrine Therapy

Patients with high or intermediate SET index had similar frequency of clinical node-positive status at presentation compared with low SET (12 of 22 *v* 68 of 100) and similar frequency of pathologic

Table 3. Multivariate Cox Regression Analysis of Association With DRFS

Factor	Hazard Ratio	95% CI	*P*
Tamoxifen-treated patients, validation cohort (n = 230)*			
Age (> 50 *v* ≤ 50 years)	4.97	0.68 to 36.5	.115
Nodal status (positive *v* negative)	2.76	1.45 to 5.25	.002
Tumor stage (T2-3 *v* T1)	1.85	0.88 to 3.86	.102
Histologic grade (2 or 3 *v* 1)	1.37	0.52 to 3.61	.519
Allred score ER IHC (≤ 6 *v* 7 or 8)	1.20	0.65 to 2.20	.559
SET class (low or intermediate *v* high)	3.65	1.12 to 11.90	.032
T/FAC chemotherapy followed by tamoxifen and/or aromatase inhibition (n = 122)†			
Residual cancer burden (continuous)	2.07	1.20 to 3.60	.009
SET index (continuous)	0.19	0.05 to 0.69	.011
Interaction term (RCB × SET)	1.49	0.99 to 2.24	.054

NOTE. The hazard ratio is a measure of the risk of distant relapse or death.

Abbreviations: DRFS, distant relapse-free survival; ER, estrogen receptor; IHC, immunohistochemistry; SET, sensitivity to endocrine therapy; T/FAC, paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide; RCB, residual cancer burden.

*Sixty-eight cases were removed from the multivariate analysis of the tamoxifen validation cohort due to partially missing data. Likelihood ratio test for the addition of SET class was 6.57 on 1 *df*, $P = .010$.

†Likelihood ratio test for the addition of SET index and interaction term was 8.45 on 2 *df*, $P = .015$.

response from neoadjuvant chemotherapy compared with low SET (pathologic complete response in three of 22 *v* five of 100, pCR or RCB-I in six of 22 *v* 35 of 100; χ^2 tests not significant). Despite this, point estimates of DRFS were 100% (95% CI, 100% to 100%) for high or intermediate, and 82.4% (95% CI, 75.1% to 90.4%) for low SET index categories at 5 years of follow-up (Fig 3C). Both the pathologic response from chemotherapy (RCB index) and the SET index of the tumor at the time of diagnosis were independently predictive of distant relapse risk, and their interaction term was also borderline significant (Table 3). Graphical plots to illustrate this interaction (Fig 3D; Data Supplement) demonstrate that elevated endocrine sensitivity (SET index) seems to be associated with reduced relapse risk when there is less than extensive RCB after chemotherapy and particularly when RCB is low.

DISCUSSION

This study demonstrates and confirms that SET index (a measure of transcriptional activity related to ER) is predictive of DRFS in tamoxifen-treated patients (Table 3, Figs 1 and 2) but is not prognostic in untreated patients (Figs 3A and 3B). We acknowledge there were different age distributions for treated and untreated cohorts (Table 2) because sample collection included an era when the role of adjuvant tamoxifen therapy had not yet been defined for premenopausal women.[27] Nevertheless, SET index can be used to estimate DRFS if a patient were to receive adjuvant endocrine therapy alone and in conjunction with other clinicopathologic information to determine whether or not additional treatment might be indicated to further improve the likelihood of cure. For example, patients with node-negative breast cancer and high SET index could reasonably select a standard adjuvant endocrine therapy alone, but others might benefit from additional treatment including chemotherapy or investigational treatments (Fig 2C).

Lymph node status was independently prognostic in the tamoxifen-treated patients (Table 3, Figs 2C and 2D).[28] Therefore, it is important to consider whether chemotherapy should be encouraged for patients with node-positive and ER-positive breast cancer or whether a predictive test for endocrine sensitivity could identify node-positive patients with either excellent survival from endocrine therapy alone or for whom added chemotherapy is futile and novel therapies are needed. In two recent reports, patients with node-positive and ER-positive breast cancer had clinically significant (> 10%) risk of relapse for any 21-gene recurrence score class.[29,30] In one study, low or intermediate recurrence score identified a subset for whom chemotherapy offered no significant benefit over tamoxifen alone, but recurrence score failed to identify any subset with excellent survival from either treatment arm.[29] We note that SET index also failed to identify a node-positive subset with less than 20% risk of distant relapse from adjuvant tamoxifen alone (Fig 2D).

SET index did identify patients with high or intermediate SET index who had excellent survival with T/FAC chemotherapy followed by endocrine therapy (Fig 3C). The endocrine predictive utility of SET index was independent of pathologic response from chemotherapy (Table 3). Furthermore, it seems (Fig 3D) that elevated SET index was more strongly associated with reduced relapse risk if there had been some response to prior T/FAC chemotherapy. However, the prognosis of those with chemo-resistant disease (high RCB) remained poor, irrespective of endocrine sensitivity (SET). This supports our interpretation of SET index as an endocrine therapy predictor and also demonstrates that partial or better response to chemotherapy in a tumor with intrinsic endocrine sensitivity can facilitate further benefit from adjuvant endocrine therapy (sequential synergy).

The results of this study challenge a popular view that chemosensitive tumors and endocrine-sensitive tumors tend to be mutually exclusive within ER-positive breast cancer.[4,31,32] This view may depend on how chemosensitivity and endocrine sensitivity are predicted. For example, genomic tests that result from empirical methods to train signatures on molecular class or on survival status at a specified time-point after diagnosis tend to rely heavily on measurement of proliferation as an essential prognostic and predictive component.[20,29,33-36] Proliferation has complex associations with outcome, being associated with poor prognosis and greater likelihood of pathologic response but also with higher risk of distant relapse after chemotherapy, and for neoadjuvant endocrine therapy is only predictive of benefit if suppressed after exposure to preoperative endocrine therapy (pharmacodynamic response).[14,15,18,35,37] In contrast, SET

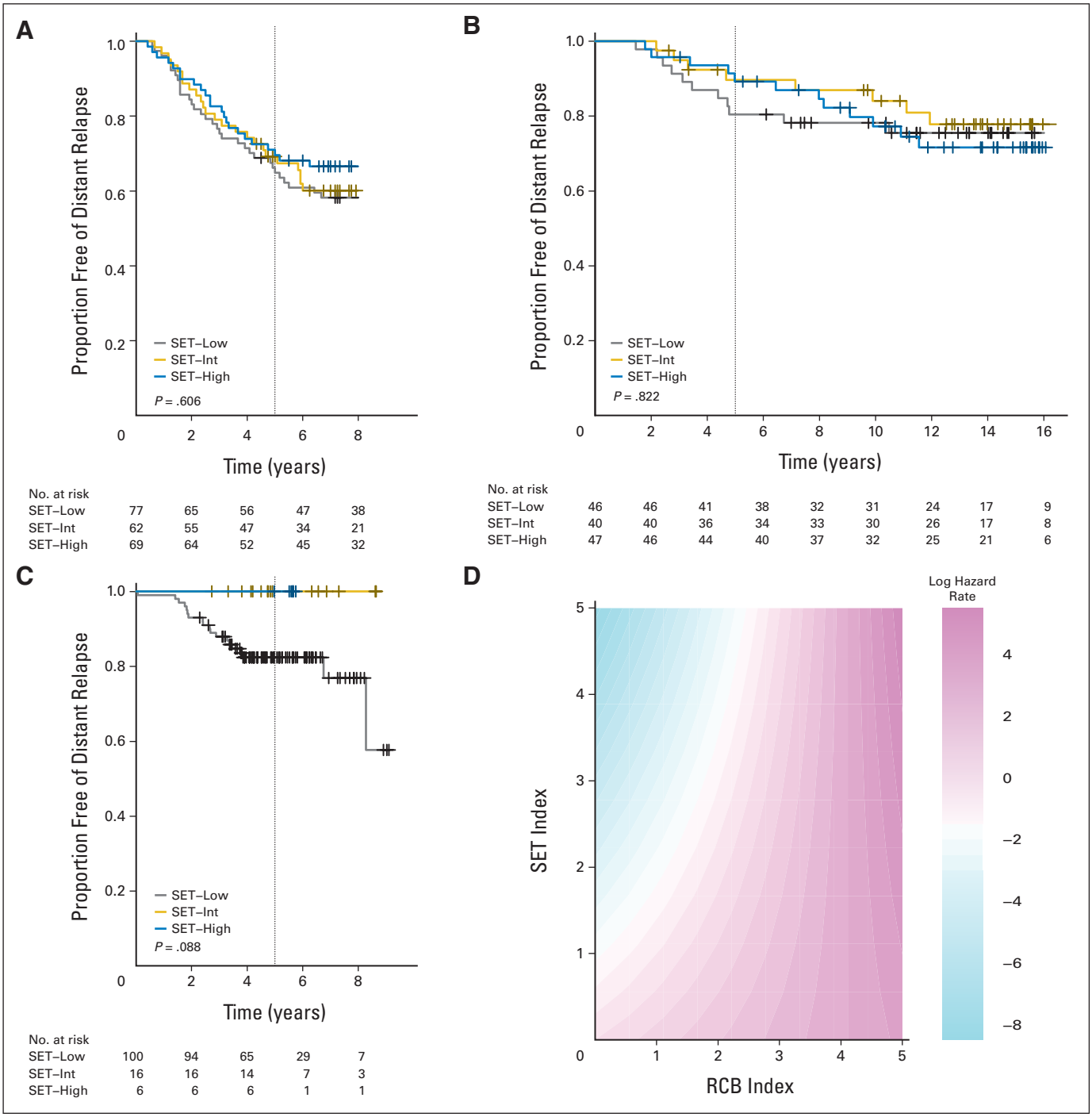

Fig 3. Kaplan-Meier estimates of relapse-free survival in (A, B) two cohorts of estrogen receptor (ER) –positive, node-negative patients who did not receive any prior hormonal therapy, and (C) in patients with clinically higher-risk ER-positive breast cancer who received neoadjuvant chemotherapy (paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide) followed by adjuvant endocrine therapy. Endocrine sensitivity groups were defined by the SET index. *P* values are from the log-rank test. (D) Contour plot depicting the dependence of the hazard rate of distant relapse or death on residual cancer burden after neoadjuvant chemotherapy (RCB index) and endocrine sensitivity (SET index) according to the Cox regression model of Table 3 estimated from the cohort in (C).

index was conceptually derived to address a targeted transcriptional pathway, has less reliance on proliferation genes, and so is probably less subject to the mixed effects of prognosis, chemosensitivity and endocrine sensitivity that would be variably represented in a single composite result from other empirically derived signatures.

We recognize that efforts to directly compare the performance of SET index with other genomic signatures would be severely limited by technical biases. For example, although there was no association between a microarray-based approximation of recurrence score and relapse risk in the second validation cohort (Data Supplement), this score has not been validated against the commercial reverse transcriptase polymerase chain reaction assay.[15,36] Furthermore, the presence of systematic bias in SET index values between two different versions of Affymetrix U133 microarrays that have identical

oligonucleotide probe sequences (Data Supplement) is cautionary for meta-analyses of microarray data derived using different technical methods.

In this study, approximately 25% of patients with ER-positive node-negative breast cancer had high SET index values and excellent survival from 5 years of endocrine therapy alone. Another 30% of patients with intermediate SET index values might consider sequential chemo-endocrine therapy or prolonged and different endocrine therapy, and the remaining 25% to 50% with low SET index are best advised to consider chemo-endocrine therapy or a clinical trial. In addition, approximately 20% of patients with clinical stage II or III disease had high or intermediate SET index and excellent 5-year DRFS that was independent of their chemotherapy response, but attributable to sequential benefits from chemo-endocrine therapy. We expect that additional future studies that evaluate the predictive performance of SET index would further inform this clinical interpretation.

The clinical relevance of independent prediction of endocrine sensitivity is unlikely to be based on a single test result interpreted in isolation and should not dissuade the use of adjuvant endocrine therapy as a standard treatment for any eligible patient. Rather, SET index results would be better interpreted in the context of nodal status and combined with independent tests for prognosis and chemosensitivity. Reducing the complex puzzle of adjuvant treatment to its main components, with separate tests for each (prognosis and prediction of benefit from endocrine, chemotherapy, and targeted therapies), should become more effective than any single composite test result in realizing the potential of multiplex technologies to provide the next generation of diagnostic tools for personalized cancer treatment planning.[35,38] In that context, SET index would be considered as one important piece of a diagnostic puzzle for personalized breast cancer treatment.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception and design: W. Fraser Symmans, Christos Hatzis, Lajos Pusztai

Financial support: W. Fraser Symmans, Christos Sotiriou, Daniel J. Booser, Lajos Pusztai

Administrative support: W. Fraser Symmans, Christos Sotiriou, Fabrice Andre, Florentia Peintinger, Peter Regitnig, Christian Marth, Suzette Delaloge, Vicente Valero, Gabriel N. Hortobagyi

Provision of study materials or patients: Christos Sotiriou, Fabrice Andre, Florentia Peintinger, Peter Regitnig, Guenter Daxenbichler, Christine Desmedt, Julien Domont, Christian Marth, Suzette Delaloge

Collection and assembly of data: W. Fraser Symmans, Christos Sotiriou, Peter Regitnig, Guenter Daxenbichler, Christine Desmedt, Julien Domont, Thomas Bauernhofer

Data analysis and interpretation: W. Fraser Symmans, Christos Hatzis, Lajos Pusztai

Manuscript writing: W. Fraser Symmans, Christos Hatzis, Christos Sotiriou, Fabrice Andre, Florentia Peintinger, Peter Regitnig, Guenter Daxenbichler, Christine Desmedt, Julien Domont, Christian Marth, Suzette Delaloge, Thomas Bauernhofer, Vicente Valero, Daniel J. Booser, Gabriel N. Hortobagyi, Lajos Pusztai

Final approval of manuscript: W. Fraser Symmans, Christos Hatzis, Christos Sotiriou, Fabrice Andre, Florentia Peintinger, Peter Regitnig, Guenter Daxenbichler, Christine Desmedt, Julien Domont, Christian Marth, Suzette Delaloge, Thomas Bauernhofer, Vicente Valero, Daniel J. Booser, Gabriel N. Hortobagyi, Lajos Pusztai

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Glossary Terms

Gene expression profile: The expression of a set of genes in a biologic sample (eg, blood, tissue) using microarray, RT-PCR, or other technology capable of measuring gene expression.

Validation: The process that tests the performance of a previously defined classifier or prognostic model on a new set of patients. For example, a gene expression signature classifier developed using data from one set of patients might be validated on another, independent set of patients.

Recurrence score: The Recurrence Score is a number between 0 and 100 that corresponds to a specific likelihood of breast cancer recurrence within 10 years of initial diagnosis. The score is derived from a mathematical function combining the expression values of 16 breast cancer–related genes and five reference genes.

RT-PCR (reverse-transcriptase polymerase chain reaction): PCR is a method that allows logarithmic amplification of short DNA sequences within a longer, double-stranded DNA molecule. Gene expression can be measured after extraction of total RNA and preparation of cDNA by a reverse-transcription step. Thus, RT-PCR enables the detection of PCR products on a real-time basis, making it a sensitive technique for quantitating changes in gene expression.

Oligonucleotide arrays: High-density arrays containing in situ synthesized antisense oligonucleotides (an average of 25 bases long) matching thousands of mRNA transcripts sequences.

SET (sensitivity to endocrine therapy) index: A multigene expression profile that was developed to measure estrogen receptor–related transcription in breast cancer.

RCB (residual cancer burden): An index to estimate the extent of residual invasive cancer in the breast and regional lymph nodes after neoadjuvant chemotherapy. RCB combines the following parameters derived from the review of routine pathology materials: two-dimensional extent of residual primary tumor, proportion of this primary tumor area that contains cancer cells, proportion of the residual primary cancer that is in situ, the number of involved regional lymph nodes, and the diameter of the largest nodal metastasis.