

The New England Journal of Medicine

Copyright © 2002 by the Massachusetts Medical Society

VOLUME 347

DECEMBER 19, 2002

NUMBER 25



A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

MARC J. VAN DE VIJVER, M.D., PH.D., YUDONG D. HE, PH.D., LAURA J. VAN 'T VEER, PH.D., HONGYUE DAI, PH.D.,
AUGUSTINUS A.M. HART, M.Sc., DORIEN W. VOSKUIL, PH.D., GEORGE J. SCHREIBER, M.Sc., JOHANNES L. PETERSE, M.D.,
CHRIS ROBERTS, PH.D., MATTHEW J. MARTON, PH.D., MARK PARRISH, DOUWE ATSMAN, ANKE WITTEVEEN,
ANNUSKA GLAS, PH.D., LEONIE DELAHAYE, TONY VAN DER VELDE, HARRY BARTELINK, M.D., PH.D.,
SJOERD RODENHUIS, M.D., PH.D., EMIEL T. RUTGERS, M.D., PH.D., STEPHEN H. FRIEND, M.D., PH.D.,
AND RENÉ BERNARDS, PH.D.

ABSTRACT

Background A more accurate means of prognostication in breast cancer will improve the selection of patients for adjuvant systemic therapy.

Methods Using microarray analysis to evaluate our previously established 70-gene prognosis profile, we classified a series of 295 consecutive patients with primary breast carcinomas as having a gene-expression signature associated with either a poor prognosis or a good prognosis. All patients had stage I or II breast cancer and were younger than 53 years old; 151 had lymph-node-negative disease, and 144 had lymph-node-positive disease. We evaluated the predictive power of the prognosis profile using univariable and multivariable statistical analyses.

Results Among the 295 patients, 180 had a poor-prognosis signature and 115 had a good-prognosis signature, and the mean (\pm SE) overall 10-year survival rates were 54.6 ± 4.4 percent and 94.5 ± 2.6 percent, respectively. At 10 years, the probability of remaining free of distant metastases was 50.6 ± 4.5 percent in the group with a poor-prognosis signature and 85.2 ± 4.3 percent in the group with a good-prognosis signature. The estimated hazard ratio for distant metastases in the group with a poor-prognosis signature, as compared with the group with the good-prognosis signature, was 5.1 (95 percent confidence interval, 2.9 to 9.0; $P < 0.001$). This ratio remained significant when the groups were analyzed according to lymph-node status. Multivariable Cox regression analysis showed that the prognosis profile was a strong independent factor in predicting disease outcome.

Conclusions The gene-expression profile we studied is a more powerful predictor of the outcome of disease in young patients with breast cancer than standard systems based on clinical and histologic criteria. (N Engl J Med 2002;347:1999-2009.)

Copyright © 2002 Massachusetts Medical Society.

ADJUVANT systemic therapy substantially improves disease-free and overall survival in both premenopausal and postmenopausal women up to the age of 70 years with lymph-node-negative or lymph-node-positive breast cancer.^{1,2} It is generally agreed that patients with poor prognostic features benefit the most from adjuvant therapy.^{3,4} The main prognostic factors in breast cancer are age, tumor size, status of axillary lymph nodes, histologic type of the tumor, pathological grade, and hormone-receptor status. A large number of other factors have been investigated for their potential to predict the outcome of disease, but in general, they have only limited predictive power.⁵

Using complementary DNA (cDNA) microarrays to analyze breast-cancer tissue, Perou et al. identified tumors with distinct patterns of gene expression that they termed "basal type" and "luminal type."⁶ These subgroups differ with respect to the outcome of disease in patients with locally advanced breast cancer.⁷ In addition, microarray analysis has been used to distinguish cancers associated with *BRCA1* or *BRCA2* mutations^{8,9} and to determine estrogen-receptor status^{6,9,10} and lymph-node status.^{11,12}

Using inkjet-synthesized oligonucleotide microarrays, we recently identified a gene-expression profile

From the Divisions of Diagnostic Oncology (M.J.V., L.J.V., D.W.V., J.L.P., D.A., A.W., A.G., L.D.), Radiotherapy (A.A.M.H., H.B.), Medical Oncology (S.R.), Biometrics (T.V.), Surgical Oncology (E.T.R.), and Molecular Carcinogenesis (R.B.), Netherlands Cancer Institute, Amsterdam; the Center for Biomedical Genetics, Amsterdam (R.B.); and Rosetta Inpharmatics, Kirkland, Wash. (Y.D.H., H.D., G.J.S., C.R., M.J.M., M.P., S.H.E.). Address reprint requests to Dr. Bernards at the Division of Molecular Carcinogenesis, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands, or at r.bernards@nki.nl.

Drs. van de Vijver, He, and van 't Veer contributed equally to this article.

that is associated with prognosis in patients with breast cancer.⁹ We analyzed only tumors that were less than 5 cm in diameter from lymph-node–negative patients who were younger than 55 years of age. We found that a classification system based on 70 genes outperformed all clinical variables in predicting the likelihood of distant metastases within five years. We estimated that the odds ratio for metastases among tumors with a gene signature associated with a poor prognosis, as compared with those having a signature associated with a good prognosis, was approximately 15 using a cross-validation procedure. Even though these results were encouraging, a limitation of the study was that the results were derived from and evaluated in two groups of patients selected on the basis of outcome: distant metastases had developed in one group within five years, and the other group remained disease-free for at least five years. Therefore, to provide a more accurate estimate of the risks of metastases associated with the two gene-expression signatures and to substantiate that the gene-expression profile of breast cancer is a clinically meaningful tool, we studied a cohort of 295 young patients with breast cancer, some of whom were lymph-node–negative and some of whom were lymph-node–positive.

METHODS

Selection of Patients

Tumors from a series of 295 consecutive women with breast cancer were selected from the fresh-frozen–tissue bank of the Netherlands Cancer Institute according to the following criteria: the tumor was primary invasive breast carcinoma that was less than 5 cm in diameter at pathological examination (pT1 or pT2); the axillary lymph nodes were tumor-negative, as determined by a biopsy of the infracavicular lymph nodes; the age at diagnosis was 52 years or younger; the calendar year of diagnosis was between 1984 and 1995; and there was no previous history of cancer, except nonmelanoma skin cancer. All patients had been treated by modified radical mastectomy or breast-conserving surgery, including dissection of the axillary lymph nodes, followed by radiotherapy if indicated. Among the 295 patients, 151 had lymph-node–negative disease (results on pathological examination, pN0) and 144 had lymph-node–positive disease (pN+). Ten of the 151 patients who had lymph-node–negative disease and 120 of the 144 who had lymph-node–positive dis-

ease had received adjuvant systemic therapy consisting of chemotherapy (90 patients), hormonal therapy (20), or both (20). Sixty-one of the patients with lymph-node–negative disease were also part of the previous study used to establish the prognosis profile.⁹ All patients were assessed at least annually for a period of at least five years. Follow-up information was extracted from the medical registry of the Netherlands Cancer Institute. The median duration of follow-up was 7.8 years (range, 0.05 to 18.3) for the 207 patients without metastasis as the first event and 2.7 years (range, 0.3 to 14.0) for the 88 patients with metastasis as the first event. The median follow-up among all 295 patients was 6.7 years (range, 0.05 to 18.3). There were no missing data. The study was approved by the medical-ethics committee of the Netherlands Cancer Institute.

Clinicopathological variables were determined as described previously.⁹ The level of expression of estrogen receptors was estimated on the basis of the hybridization results on the microarray experiments, which is a reliable assay for estrogen-receptor status.⁹ On the basis of this assay, there were 69 estrogen-receptor–negative tumors (defined by an intensity ratio of less than -0.65 U on a logarithmic scale, corresponding to staining of less than 10 percent of nuclei on immunohistochemical analysis) and 226 estrogen-receptor–positive tumors in the cohort. The histologic grade was assessed according to the method described by Elston and Ellis¹³; vascular invasion was assessed as absent, minor (one to three vessels), or major (more than three vessels).

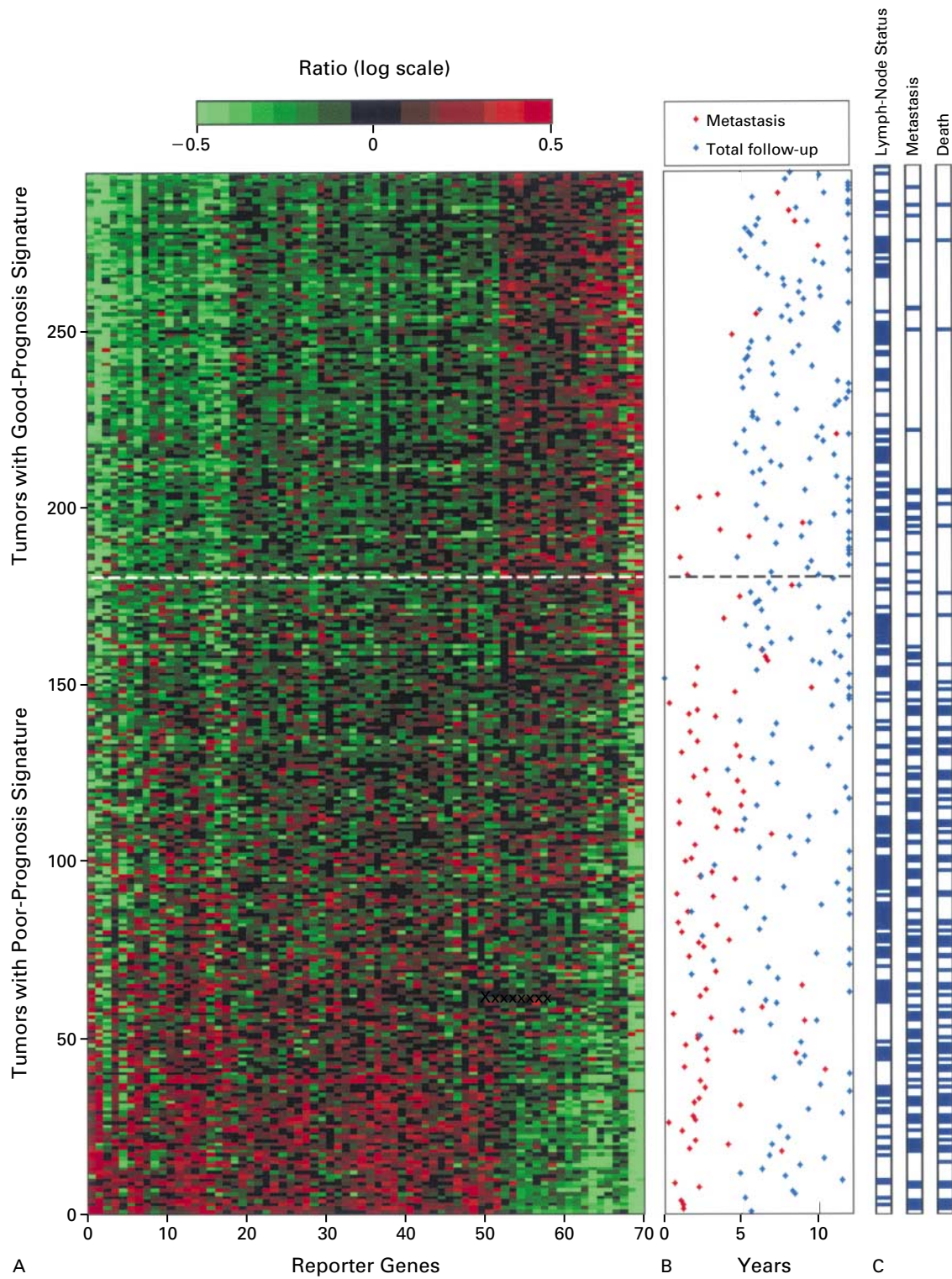
Isolation of RNA and Microarray Expression Profiling

The isolation of RNA, labeling of complementary RNA (cRNA), hybridization of labeled cRNA to 25,000-gene arrays, and assessment of expression ratios were all performed as previously described.^{9,14} In brief, tumor material was snap-frozen in liquid nitrogen within one hour after surgery. Frozen sections were stained with hematoxylin and eosin; only samples that had more than 50 percent tumor cells were selected. Thirty 30- μ m sections were used for the isolation of RNA. Total RNA was isolated with RNeasy spin columns, the RNA was then dissolved in RNase-free water. Then 25 μ g of total RNA was treated with DNase with use of the Qiagen RNeasy spin columns, the RNA was then dissolved in RNase-free water to a final concentration of 0.2 μ g per microliter, and cRNA was generated by in vitro transcription with the use of T7 RNA polymerase and 5 μ g of total RNA and labeled with Cy3 or Cy5 (Cy Dye, Amersham Pharmacia Biotech). Five micrograms of Cy-labeled cRNA from one breast-cancer tumor was mixed with the same amount of reverse-color Cy-labeled product from a pool that consisted of an equal amount of cRNA from each patient.

Labeled cRNAs were fragmented to an average size of approximately 50 to 100 nucleotides by heating the samples to 60°C in the presence of 10 mM zinc chloride and adding a hybridization buffer containing 1 M sodium chloride, 0.5 percent sodium sarcosine, 50 mM morpholino-ethane sulfonic acid (pH 6.5), and formamide

Figure 1 (facing page). Pattern of Expression of Genes Used to Determine the Prognosis and Clinical Characteristics of 295 Patients with Breast Cancer.

Panel A shows the pattern of expression of the 70 marker genes (also referred to as prognosis-classifier genes⁹) in a series of 295 consecutive patients with breast carcinomas. Each row represents the prognostic profile of the 70 marker genes for one tumor, and each column represents the relative level of expression of one gene. The tumors are numbered from 1 to 295 on the y axis, and the genes are numbered from 1 to 70 on the x axis. The genes in the horizontal direction are arrayed in the same order as in our previous study.⁹ Red indicates a high level of expression of messenger RNA (mRNA) in the tumor, as compared with the reference level of mRNA, and green indicates a low level of expression. The dotted line is the previously determined threshold between a good-prognosis signature and a poor-prognosis signature. Tumors are rank-ordered according to their correlation with the previously determined average profile in tumors from patients with a good prognosis. Panel B shows the time in years to distant metastases as a first event for those in whom this occurred, and the total duration of follow-up for all other patients. Panel C shows the lymph-node status (blue marks indicate lymph-node–positive disease, and white lymph-node–negative disease), the number of patients with distant metastases as a first event (blue marks), and the number of patients who died (blue marks).



(final concentration, 30 percent at 40°C); the final volume was 3 ml. The microarrays included the 24,479 biologic oligonucleotides as well as 1281 control probes. After hybridization, the slides were washed and scanned with a confocal laser scanner (Agilent Technologies). Fluorescence intensities on scanned images were quantified, and the values were corrected for the background level and normalized.

Validation Strategy

We wished to investigate the prognostic value of the gene-expression profile in a consecutive series of patients with breast cancer. We included 61 of the 78 patients with lymph-node–negative disease who were involved in the previous study that determined the 70-gene prognosis profile.⁹ Leaving them out would have resulted in selection bias, since the previous study included a disproportionately large number of patients in whom distant metastases developed within five years. We included these 61 patients in the study, but we used the “leave-one-out” cross-validated classification established in our previous study to predict the outcomes among these patients. In this approach, the classification of the left-out sample was based on its correlation with the mean levels of expression of the remaining samples from the patients with a good-prognosis signature, with the sample in question excluded from the gene-selection process.⁹ This approach minimizes to some extent the possibility of overestimating the value of the prognosis profile while it keeps the consecutive series complete. We also provide validation results taking only the new samples into account.

Correlation of the Microarray Data with the Prognosis Profile

For each of the 234 tumors from patients who were not included in the previous study, we calculated the correlation coefficient of the level of expression of the 70 genes with the previously determined average profile of these genes in tumors from patients with a good prognosis (C1).⁹ A patient with a correlation coefficient of more than 0.4 (the threshold in the previous study of 78 tumors that resulted in a 10 percent rate of false negative results) was then assigned to the group with a good-prognosis signature, and all other patients were assigned to the group with a poor-prognosis signature. For the 61 patients with lymph-node–negative disease who were included in the previous study, we used a cutoff value of 0.55 (corresponding to the threshold that resulted in a 10 percent rate of false negative results in the cross-validated classification in our previous study).⁹

Study Design

Study design, patient selection, RNA isolation from tumor material, histopathological analyses, clinical annotation, and clinical interpretation were carried out at the Netherlands Cancer Institute. RNA amplification and microarray hybridization were carried out at Rosetta Inpharmatics. Bioinformatic and statistical analyses were performed jointly by authors at both locations. All raw data were available to all the investigators.

Statistical Analysis

In the analysis of the probability that patients would remain free of distant metastases, we defined distant metastases as a first event to be a treatment failure; data on all other patients were censored on the date of the last follow-up visit, death from causes other than breast cancer, the recurrence of local or regional disease, or the development of a second primary cancer, including contralateral breast cancer. Data on patients were analyzed from the date of surgery to the time of the first event or the date on which data were censored, according to the method of Kaplan and Meier, and the curves were compared with use of the log-rank test. Values are expressed as means \pm SE, calculated according to the method of Tsiatis.¹⁵

We used proportional-hazards regression analysis¹⁶ to adjust the association between the correlation coefficient (C1) and metastases for other variables. All SEs were calculated with use of the sandwich estimator.¹⁷ The histologic grade, extent of vascular invasion, and number of axillary-lymph-node metastases (0 vs. 1 to 3 or 0 vs. ≥ 4) were used as variables. The linearity of the relation between the relative hazard ratio and the diameter of the tumor, age, and level of expression of estrogen receptors was tested with use of the Wald test for nonlinear components of restricted cubic splines.¹⁸ No evidence of nonlinearity was found ($P=0.83$ for age, $P=0.75$ for tumor diameter, $P=0.65$ for the number of positive nodes, and $P=0.27$ for the level of expression of estrogen receptors). We evaluated whether the hazard ratio was proportional using the method of Grambsch and Therneau.¹⁹ In addition, we determined the difference between the relative hazard ratio before and after five years of follow-up with respect to the prognosis signature using the Wald test. All calculations were performed with the S Plus 2000 or S Plus 6 statistical package.

RESULTS

Categorization of Gene-Expression Signatures

Total RNA from each tumor was isolated and used to generate cRNA, which was labeled and hybridized to microarrays containing approximately 25,000 hu-

TABLE 1. ASSOCIATION BETWEEN CLINICAL CHARACTERISTICS AND THE PROGNOSIS SIGNATURE.

CHARACTERISTIC	POOR-PROGNOSIS	GOOD-PROGNOSIS	P VALUE
	SIGNATURE (N=180)	SIGNATURE (N=115)	
	no. of patients (%)		
Age			<0.001
<40 yr	52 (29)	11 (10)	
40–44 yr	41 (23)	44 (38)	
45–49 yr	55 (31)	43 (37)	
≥50 yr	32 (18)	17 (15)	
No. of positive nodes			0.60
0	91 (51)	60 (52)	
1–3	63 (35)	43 (37)	
≥4	26 (14)	12 (10)	
Tumor diameter			0.012
≤20 mm	84 (47)	71 (62)	
>20 mm	96 (53)	44 (38)	
Histologic grade			<0.001
I (good)	19 (11)	56 (49)	
II (intermediate)	56 (31)	45 (39)	
III (poor)	105 (58)	14 (12)	
Vascular invasion			0.38
Absent	108 (60)	77 (67)	
1–3 Vessels	18 (10)	12 (10)	
>3 Vessels	54 (30)	26 (23)	
Estrogen-receptor status			<0.001
Negative	66 (37)	3 (3)	
Positive	114 (63)	112 (97)	
Surgery			0.63
Breast-conserving therapy	97 (54)	64 (56)	
Mastectomy	83 (46)	51 (44)	
Chemotherapy			0.79
No	114 (63)	71 (62)	
Yes	66 (37)	44 (38)	
Hormonal therapy			0.63
No	157 (87)	98 (85)	
Yes	23 (13)	17 (15)	

man genes.⁹ Fluorescence intensities of scanned images were quantified and normalized. We calculated the ratio of these values to the intensity of a reference pool made up of equal amounts of cRNA from all tumors. The gene-expression ratios of the previously determined 70 marker genes for all 295 tumors in this study are shown in Figure 1A. The 115 tumors with values

above the previously determined threshold⁹ were assigned to the good-prognosis category, and the 180 below the threshold were assigned to the poor-prognosis category. Figure 1B shows the time to distant metastases as a first event as well as the total duration of follow-up for all patients who did not have distant metastases as a first event. Figure 1C shows lymph-

TABLE 2. ODDS RATIO FOR DISTANT METASTASES WITHIN FIVE YEARS AS A FIRST EVENT, ACCORDING TO THE PROGNOSIS SIGNATURE.

GROUP*	NO. OF PATIENTS	DISTANT METASTASES		ODDS RATIO (95% CI)†	P VALUE‡
		WITHIN 5 YR	DISEASE-FREE >5 YR		
		no. of patients			
Patients with lymph-node–negative disease					
Patients in previous study	78			15.0 (3.3–56)	<0.001
Poor-prognosis signature		31	18		
Good-prognosis signature		3	26		
Consecutive series (new patients only)§	67			15.3 (1.8–127)	0.003
Poor-prognosis signature		11	23		
Good-prognosis signature		1	32		
Patients with lymph-node–positive disease					
Consecutive series	113			13.7 (3.1–61)	<0.001
Poor-prognosis signature		28	42		
Good-prognosis signature		2	41		
All new patients in the consecutive series	180			14.6 (4.3–50)	<0.001
Poor-prognosis signature		39	65		
Good-prognosis signature		3	73		

*The patients selected either had had distant metastases as a first event within five years or had remained free of disease for at least five years.

†Odds ratios were calculated with use of a two-by-two contingency table. CI denotes confidence interval.

‡P values were calculated with use of Fisher's exact test.

§In this analysis, patients who were part of the previous study of gene-expression profiling were excluded from the series of consecutive patients.

TABLE 3. RATE OF OVERALL SURVIVAL AND THE PROBABILITY THAT PATIENTS WOULD REMAIN FREE OF DISTANT METASTASES AT 5 AND 10 YEARS, ACCORDING TO THE PROGNOSIS SIGNATURE.*

GROUP	NO. OF PATIENTS	FREE OF DISTANT METASTASES		OVERALL SURVIVAL	
		5 YR	10 YR	5 YR	10 YR
		percent			
All patients					
Good-prognosis signature	115	94.7±2.1	85.2±4.3	97.4±1.5	94.5±2.6
Poor-prognosis signature	180	60.5±3.8	50.6±4.5	74.1±3.3	54.6±4.4
Patients with lymph-node–negative disease					
Good-prognosis signature	60	93.4±3.2	86.8±4.8	96.7±2.3	96.7±2.3
Poor-prognosis signature	91	56.2±5.5	44.1±6.3	71.5±4.8	49.6±6.1
Patients with lymph-node–positive disease					
Good-prognosis signature	55	95.2±2.6	82.7±7.8	98.2±1.8	92.0±4.8
Poor-prognosis signature	89	66.3±5.2	56.7±6.4	76.5±4.6	59.5±6.3

*Distant metastasis was a first event. Plus–minus values are means ±SE.

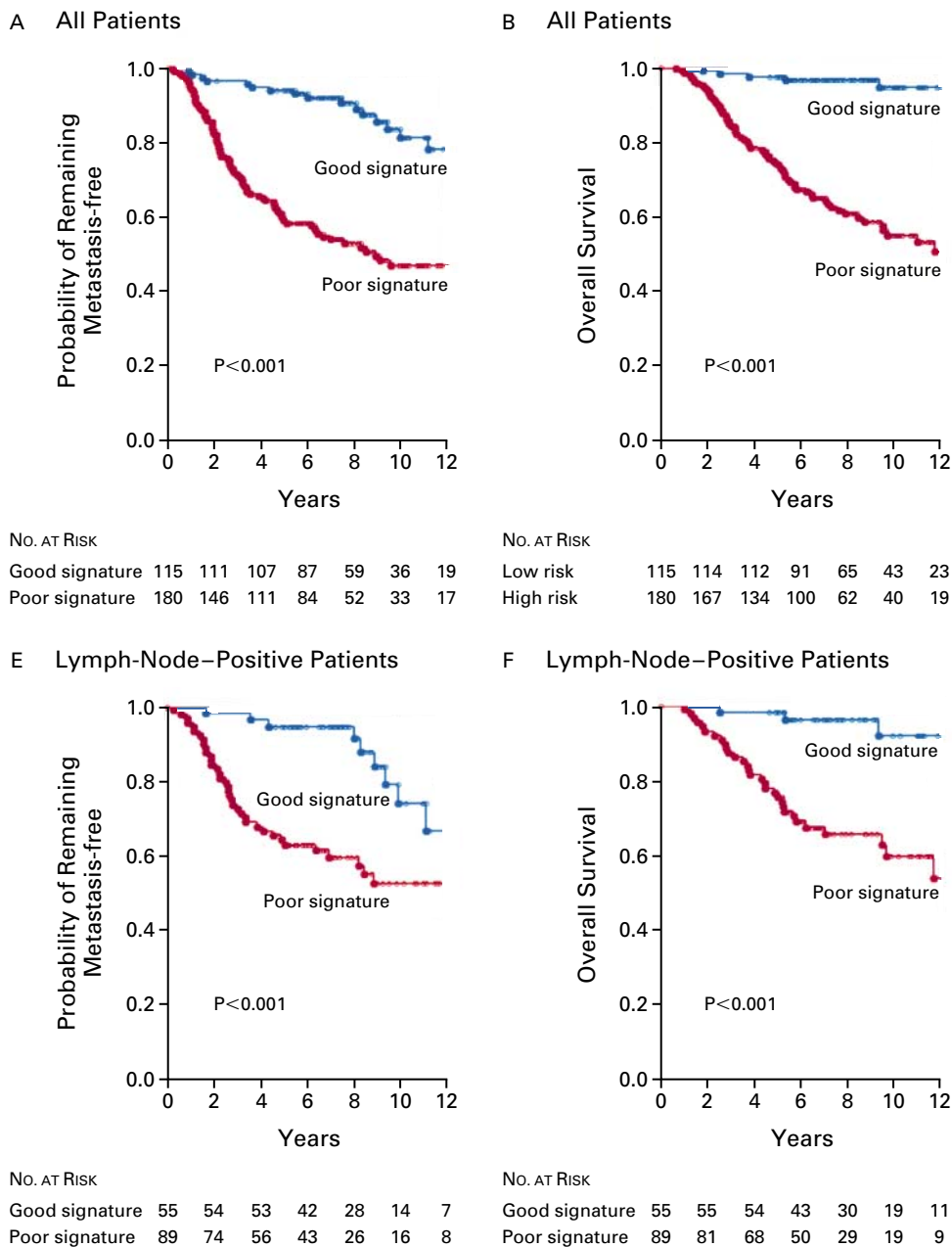
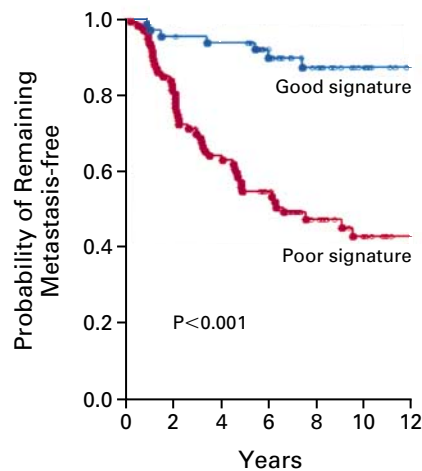


Figure 2. Kaplan–Meier Analysis of the Probability That Patients Would Remain Free of Distant Metastases and the Probability of Overall Survival among All Patients (Panels A, and B, Respectively), Patients with Lymph-Node–Negative Disease (Panels C and D [Facing Page], Respectively), and Patients with Lymph-Node–Positive Disease (Panels E and F, Respectively), According to Whether They Had a Good-Prognosis or a Poor-Prognosis Signature.

The P values were calculated with use of the log-rank test.

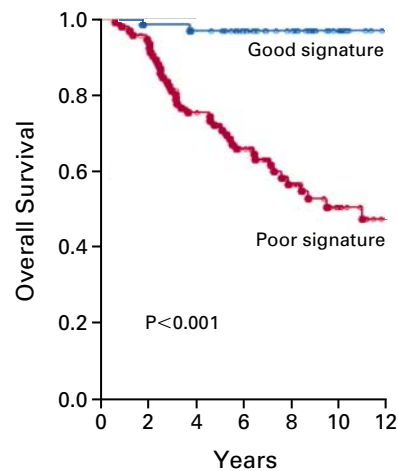
C Lymph-Node–Negative Patients



No. AT RISK

Good signature	60	57	54	45	31	22	12
Poor signature	91	72	55	41	26	17	9

D Lymph-Node–Negative Patients



No. AT RISK

Good signature	60	59	58	48	35	24	12
Poor signature	91	86	66	50	33	21	10

node status, distant-metastases status, and overall survival for all 295 patients. By comparing Figures 1A, 1B, and 1C, it can be seen that there is a strong correlation between the good-prognosis signature and the absence of (early) distant metastases or death. The patients with lymph-node–negative disease and those with lymph-node–positive disease were evenly distributed in the two groups, indicating that the prognosis profile is independent of lymph-node status. Table 1, which summarizes the association between the prognosis profile and clinical variables, shows that the prognosis profile was significantly associated with the histologic grade of the tumor ($P<0.001$), the estrogen-receptor status ($P<0.001$), and age ($P<0.001$), but not with the diameter of the tumor, the extent of vascular invasion, the number of positive lymph nodes, or treatment.

Prognostic Value of Gene-Expression Signature

In our previous study,⁹ the prognosis profile was determined in a selected group of patients with lymph-node–negative disease. In the current study, we evaluated both patients with lymph-node–negative disease and patients with lymph-node–positive disease. To validate our previous finding, we first calculated the estimated odds ratio for the development of metastases within five years for the patients with lymph-node–negative disease in the present series (thus excluding the 61 patients who were also part of the previous study⁹) (Table 2). This analysis included only patients in whom distant metastases developed within five years and patients who remained disease-free for at least five years. The odds ratio for the development of dis-

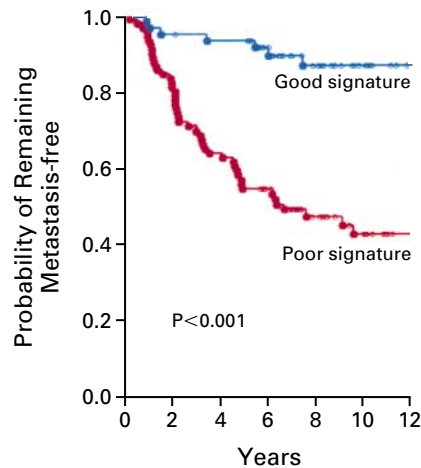
tant metastases within five years in this group was similar to the ratio in our previous study (15.3 and 15, respectively) (Table 2). The prognosis signature was also highly predictive of the risk of distant metastases among the subgroup of patients with lymph-node–positive disease and among the subgroup of all new patients (Table 2). These results highlight the value of

TABLE 4. MULTIVARIABLE PROPORTIONAL-HAZARDS ANALYSIS OF THE RISK OF DISTANT METASTASES AS A FIRST EVENT.

VARIABLE	HAZARD RATIO (95% CI)*	P VALUE
Poor-prognosis signature (vs. good-prognosis signature)	4.6 (2.3–9.2)	<0.001
Age (per 10-yr increment)	0.73 (0.50–1.06)	0.10
Lymph-node status (per positive node)	1.13 (1.03–1.24)	0.01
Diameter of tumor (per cm)	1.56 (1.22–2.0)	<0.001
Tumor grade		0.54
Grade 2 (vs. grade 1)	1.35 (0.61–3.0)	
Grade 3 (vs. grade 1)	1.03 (0.44–2.4)	
Vascular invasion		0.05
1–3 Vessels (vs. 0 vessels)	0.66 (0.30–1.44)	
>3 Vessels (vs. 0 vessels)	1.65 (0.98–2.8)	
Estrogen-receptor expression (per point)†	0.86 (0.56–1.31)	0.48
Mastectomy (vs. breast-conserving therapy)	1.27 (0.79–2.0)	0.32
Chemotherapy (vs. no chemotherapy)	0.37 (0.20–0.66)	<0.001
Hormonal treatment (vs. no hormonal treatment)	0.62 (0.29–1.34)	0.23

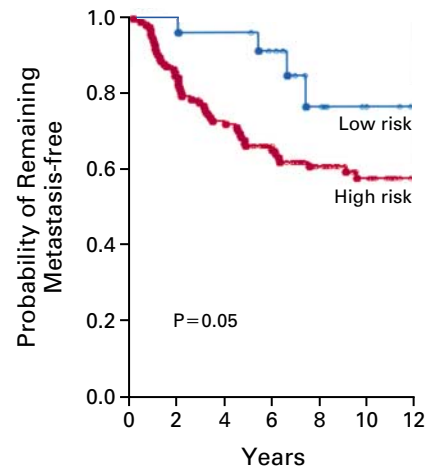
*CI denotes confidence interval.

†The log ratio of estrogen-receptor expression was used as a continuous variable.

A Gene-Expression Profiling

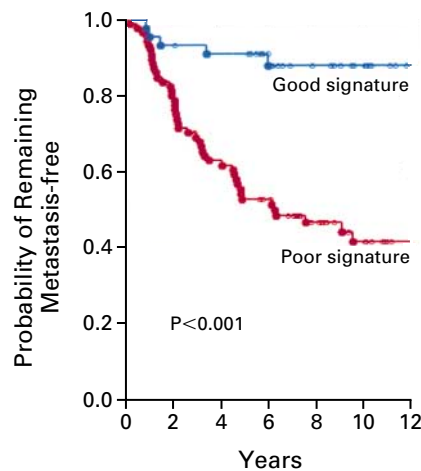
No. AT RISK

Good signature	60	57	54	45	31	22	12
Poor signature	91	72	55	41	26	17	9

B St. Gallen Criteria

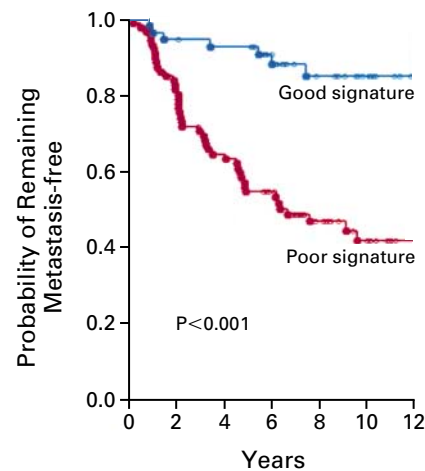
No. AT RISK

Low risk	22	22	21	17	9	5	2
High risk	129	107	88	69	48	34	19

D St. Gallen, High Risk

No. AT RISK

Good signature	43	40	37	31	23	18	10
Poor signature	86	67	51	38	25	16	9

E NIH, High Risk

No. AT RISK

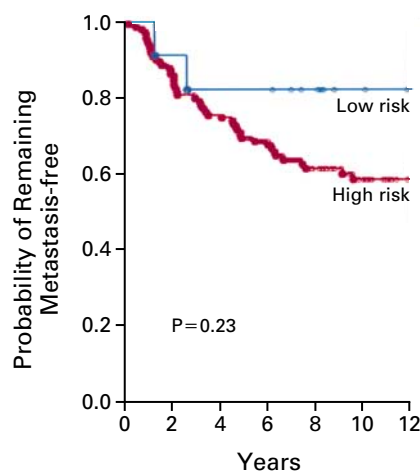
Good signature	53	50	47	38	27	21	12
Poor signature	87	69	53	39	24	16	9

the prognosis profile and the robustness of the profiling technique.

To obtain a more useful estimate of the clinical outcome, we calculated the probability of remaining free of distant metastases and overall survival according to the prognosis profile. For this analysis, we first included all 295 patients (Table 3 and Fig. 2A and 2B), even the 61 patients with lymph-node–negative disease who were in the previous study.⁹ Leaving out these patients would have resulted in selection bias,

since the first series contained a disproportionately large number of patients in whom distant metastases developed within five years. However, a different classification strategy was used for these patients, to correct for overfitting (see the Methods section). The Kaplan–Meier curves showed a significant difference in the probability that patients would remain free of distant metastases and the probability of overall survival between the group with a good-prognosis signature and the group with a poor-prognosis signature. The

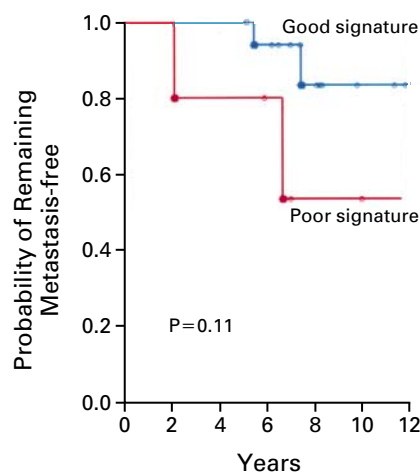
C NIH Consensus Criteria



No. AT RISK

Low risk	11	10	9	9	6	2	0
High risk	140	119	100	77	51	37	21

F St. Gallen, Low Risk



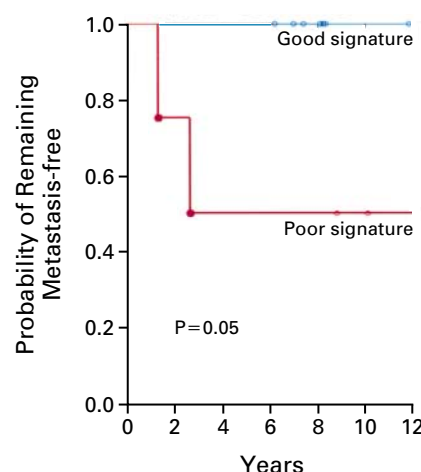
No. AT RISK

Good signature	17	17	17	14	8	4	2
Poor signature	5	5	4	3	1	1	0

Figure 3. Kaplan-Meier Analysis of the Probability That Patients Would Remain Free of Distant Metastases among 151 Patients with Lymph-Node–Negative Breast Cancer with the Use of Gene-Expression Profiling (Good-Prognosis and Poor-Prognosis Signatures) (Panel A [Facing Page]), the St. Gallen Criteria for Low-Risk and High-Risk Groups (Panel B [Facing Page]), the National Institutes of Health (NIH) Consensus Criteria for Low-Risk and High-Risk Groups (Panel C), the St. Gallen Criteria for a High-Risk Group and Gene-Expression Profiling (Panel D [Facing Page]), the NIH Criteria for a High-Risk Group and Gene-Expression Profiling (Panel E [Facing Page]), the St. Gallen Criteria for a Low-Risk Group and Gene-Expression Profiling (Panel F), and the NIH Criteria for a Low-Risk Group and Gene-Expression Profiling (Panel G).

For Panels D, E, F, and G, patients were divided into those with a good-prognosis signature and those with a poor-prognosis signature according to gene-expression profiling. The P values were calculated with use of the log-rank test.

G NIH, Low Risk



No. AT RISK

Good signature	7	7	7	7	4	1	0
Poor signature	4	3	2	2	2	1	0

estimated hazard ratio for distant metastases as a first event in the group with a poor-prognosis signature as compared with the group with a good-prognosis signature over the entire follow-up period was 5.1 (95 percent confidence interval, 2.9 to 9.0; $P < 0.001$); the prognosis profile was associated with a significantly higher hazard ratio during the first five years of follow-up (hazard ratio, 8.8; 95 percent confidence interval, 3.8 to 20; $P < 0.001$) than after five years (hazard ratio, 1.8; 95 percent confidence interval, 0.69 to 4.5;

$P = 0.24$). The hazard ratio for overall survival was 8.6 (95 percent confidence interval, 4 to 19; $P < 0.001$).

In the series of 151 patients with lymph-node–negative disease, the prognosis profile was also extremely useful in predicting the outcome of disease (Table 3 and Fig. 2C and 2D). In this group of patients, the hazard ratio for distant metastases was 5.5 among those with a poor-prognosis signature as compared with those with a good-prognosis signature (95 percent confidence interval, 2.5 to 12.2; $P < 0.001$).

The prognosis profile was also strongly associated with the outcome in the group of 144 patients with lymph-node–positive disease (Table 3 and Fig. 2E and 2F). In this group, the hazard ratio for distant metastases was 4.5 (95 percent confidence interval, 2.0 to 10.2; $P < 0.001$).

Multivariable Analysis

Table 4 shows the results of the multivariable analysis of the risk of distant metastases as the first event. The only independent predictive factors were a poor-prognosis signature, a larger diameter of the tumor, and the nonuse of adjuvant chemotherapy. During the period in which these patients were treated, most premenopausal patients with lymph-node–positive disease received adjuvant chemotherapy, whereas the majority of patients with lymph-node–negative disease did not receive adjuvant treatment. Patients who received adjuvant chemotherapy in this series had a higher likelihood of remaining free of distant metastases (hazard ratio for distant metastases, 0.37; 95 percent confidence interval, 0.20 to 0.66; $P < 0.001$). The poor-prognosis signature was by far the strongest predictor of the likelihood of distant metastases, with an overall hazard ratio of 4.6 (95 percent confidence interval, 2.3 to 9.2; $P < 0.001$).

DISCUSSION

We previously identified a gene-expression profile of 70 genes that is associated with the risk of early distant metastases in young patients with lymph-node–negative breast cancer.⁹ In the present study we tested this profile in a series of 295 consecutive patients who were treated at the hospital of the Netherlands Cancer Institute. The profile performed best as a predictor of the appearance of distant metastases during the first five years after treatment. This finding is not unexpected, since the tumors on which the profile was based had all metastasized within five years. The prognosis profile is also a strong predictor of the development of distant metastases in patients with lymph-node–positive disease. This finding is important, since the presence of lymph-node metastases is by itself a strong predictor of poor survival. Since most patients with lymph-node–positive breast cancer in our study received adjuvant chemotherapy or hormonal therapy (120 of 144 patients), we could not evaluate the prognostic value of the profile in patients with untreated lymph-node–positive disease. There is, however, no indication of an effect of adjuvant chemotherapy on the prognostic value of the profile (data not shown).

Figure 3 shows the Kaplan–Meier estimates of the probability that patients would remain free of distant metastases among the 151 patients with lymph-node–negative cancer, according to whether the patients were classified with the use of gene-expression pro-

filings (Fig. 3A), the St. Gallen criteria³ (Fig. 3B), or the National Institutes of Health (NIH) consensus criteria⁴ (Fig. 3C). The St. Gallen and NIH criteria classify patients as at low risk or high risk on the basis of various histologic and clinical characteristics. This comparison shows that the prognosis profile assigned many more patients with lymph-node–negative disease to the low-risk (good-prognosis signature) group than did the traditional methods (40 percent, as compared with 15 percent according to the St. Gallen criteria and 7 percent according to the NIH criteria). Moreover, low-risk patients identified by gene-expression profiling had a higher likelihood of metastasis-free survival than those classified according to the St. Gallen or NIH criteria, and high-risk patients identified by gene-expression profiling tended to have a higher rate of distant metastases than did the high-risk patients identified by the St. Gallen or NIH criteria. This result indicates that both sets of the currently used criteria misclassify a clinically significant number of patients. Indeed, the high-risk group defined according to the NIH criteria included many patients who had a good-prognosis signature and a good outcome (Fig. 3E). Conversely, the low-risk group identified by the NIH criteria included patients with a poor-prognosis signature and poor outcome (Fig. 3G). Similar subgroups were identified within the high-risk and low-risk groups identified according to the St. Gallen criteria (Fig. 3D and 3F, respectively). Since both the St. Gallen and the NIH subgroups contain misclassified patients (who can be better identified through the prognosis signature), these patients would be either overtreated or undertreated in current clinical practice.

Our data indicate that the ability to metastasize to distant sites is an early and inherent genetic property of breast cancer. Our findings argue against the widely accepted idea that metastatic potential is acquired relatively late during multistep tumorigenesis.²⁰ If the metastatic ability of breast cancer is determined early in tumorigenesis, early prognostic testing could be undertaken, an approach that would clearly be beneficial. On the other hand, an early onset of metastatic capability theoretically limits the benefit of early detection and treatment. Furthermore, our findings suggest that the molecular mechanism leading to hematogenous (distant) metastases is distinct from the mechanism of lymphogenic (regional) spread of tumor cells. Our conclusion that the prognosis profile is independent of lymphogenic metastases is based on its strong predictive power with respect to hematogenous metastases, regardless of the presence or absence of lymph-node involvement.

Our data indicate that classification of patients into high-risk and low-risk subgroups on the basis of the prognosis profile may be a useful means of guiding

adjuvant therapy in patients with lymph-node-positive breast cancer. This approach should also improve the selection of patients who would benefit from adjuvant systemic treatment, reducing the rate of both overtreatment and undertreatment.

Supported by grants from the Netherlands Cancer Institute. The DNA microarray hybridization was carried out by the Kirkland facility of Rosetta Inpharmatics at the company's cost.

Drs. van de Vijver, He, van 't Veer, Dai, Hart, Roberts, Friend, and Bernards are named inventors on a patent to use microarray technology to ascertain breast-cancer prognosis. Drs. He, Dai, Schreiber, Roberts, Bernards, Marton, Parrish, and Friend report having equity in Merck.

We are indebted to Arno Floore, Petra Kristel, and Carla Schippers for preparing tumor RNA; to Wil van Waardenburg, Kathy van Hees, and Otilia Dalesio for managing the medical-records data; to Aaron Benner, David Slade, John McDonald, John Koch, and the staff of the Gene Expression Laboratory of Rosetta for performing microarray experiments; and to Anton Berns, Bas Kreike, Mao Mao, Roland Stoughton, and Peter Linsley for helpful suggestions.

REFERENCES

1. Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. *Lancet* 1998;352:930-42.
2. *Idem*. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998;351:1451-67.
3. Goldhirsch A, Glick JH, Gelber RD, Coates AS, Senn HJ. Meeting highlights: International Consensus Panel on the Treatment of Primary Breast Cancer: Seventh International Conference on Adjuvant Therapy of Primary Breast Cancer. *J Clin Oncol* 2001;19:3817-27.
4. Eifel P, Axelson JA, Costa J, et al. National Institutes of Health Consensus Development Conference Statement: adjuvant therapy for breast cancer, November 1-3, 2000. *J Natl Cancer Inst* 2001;93:979-89.

5. Isaacs C, Stearns V, Hayes DF. New prognostic factors for breast cancer recurrence. *Semin Oncol* 2001;28:53-67.
6. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747-52.
7. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869-74.
8. Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. *N Engl J Med* 2001;344:539-48.
9. van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530-6.
10. Gruvberger S, Ringner M, Chen Y, et al. Estrogen receptor status in breast cancer is associated with remarkably distinct gene expression patterns. *Cancer Res* 2001;61:5979-84.
11. West M, Blanchette C, Dressman H, et al. Predicting the clinical status of human breast cancer by using gene expression profiles. *Proc Natl Acad Sci U S A* 2001;98:11462-7.
12. Ahr A, Karn T, Solbach C, et al. Identification of high risk breast-cancer patients by gene expression profiling. *Lancet* 2002;359:131-2.
13. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology* 1991;19:403-10.
14. Hughes TR, Mao M, Jones AR, et al. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol* 2001;19:342-7.
15. Klein JP. Small sample moments of the estimators of the variance of the Kaplan-Meier and Nelson-Aalen estimators. *Scand J Stat* 1991;18:333-40.
16. Cox DR. Regression models and life-tables. *J R Stat Soc [B]* 1972;34:187-220.
17. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. *J Am Stat Assoc* 1989;84:1074-8.
18. Therneau TM, Grambsch PM, Fleming TR. Martingale-based residuals for survival models. *Biometrika* 1990;77:147-60.
19. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika* 1994;81:515-26.
20. Bernards R, Weinberg RA. A progression puzzle. *Nature* 2002;418:823.

Copyright © 2002 Massachusetts Medical Society.

VIEW CURRENT JOB POSTINGS AT THE NEW NEJM CAREER CENTER

Visit our online Career Center for physicians at www.nejmjobs.org to see the new, expanded features and services available. Physicians can now conduct a quick search of the public data base by specialty and view hundreds of current openings that are updated daily online at the new Career Center.