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Prognostic factors in breast cancer: one fits all?

Mook, S.

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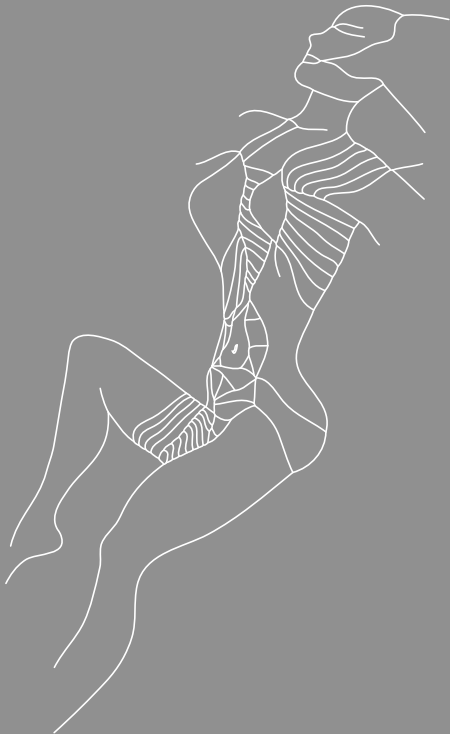
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Chapter 6

Metastatic potential of T1 breast cancer can be predicted by the 70-gene MammaPrint signature



Stella Mook*
Michael Knauer*
Jolien M. Bueno de Mesquita
Valesca P. Retel
Jelle Wesseling
Sabine C. Linn
Laura J. Van 't Veer
Emiel J.Th. Rutgers
* Contributed equally

Abstract

Background

Mammographic screening and increased awareness has led to an increase in the detection of T1 breast tumors that are generally estimated as having low risk of recurrence after locoregional treatment. However, even small tumors can metastasize, which leaves us with the question for the necessity of adjuvant treatment. Therefore, additional prognostic markers are needed to tailor adjuvant systemic treatment for these relatively low-risk patients. The aim of our study was to evaluate the accuracy of the 70-gene MammaPrint™ signature in T1 breast cancer.

Materials and Methods

We selected 964 patients from previously reported studies with pT1 tumors (≤ 2 cm). Frozen tumor samples were hybridized on the 70-gene signature array at the time of the initial study and classified as having good prognosis or poor prognosis.

Results

The median follow-up was 7.1 years (range 0.2–25.2). The 10-year distant metastasis-free (DMFS) and breast cancer specific survival (BCSS) probabilities were 87% (SE 2%) and 91% (SE 2%), respectively, for the good prognosis-signature group ($n = 525$), and 72% (SE 3%) and 72% (SE 3%), respectively, for the poor prognosis signature group ($n = 439$). The signature was an independent prognostic factor for BCSS at 10 years (multivariate hazard ratio [HR] 3.25 [95% confidence interval, CI, 1.92–5.51; $p < 0.001$]). Moreover, the 70-gene MammaPrint™ signature predicted DMFS at 10 years for 139 patients with pT1ab cancers (HR 3.45 [95% CI 1.04-11.50, $P = 0.04$]).

Conclusions

The 70-gene MammaPrint™ signature is an independent prognostic factor in patients with pT1 tumors and can help to individualize adjuvant treatment recommendation in this increasing breast cancer population.

Introduction

Primary tumor size, in addition to axillary lymph node status, is considered to be one of the most important prognostic factors in breast cancer, with small tumor size being an indicator of good prognosis.¹⁻⁵ However, even small tumors can metastasize, suggesting that the ability to metastasize is an early and inherent genetic property.^{6,7} Adjuvant treatment decisions based on tumor size alone are only moderately accurate and could result in undertreatment of T1ab and overtreatment of T1c tumors. The need for adjuvant systemic therapy after locoregional therapy for patients with small tumors is unresolved.^{8,9} Currently used treatment guidelines give different recommendations for pT1ab and pT1c tumors and often the advice 'consider chemotherapy' is given, without providing specific advice for the use of prognostic factors.¹⁰⁻¹²

With the widespread introduction of breast cancer screening programs and increased awareness, the proportion of patients presenting with small tumors is ever increasing; therefore, robust and reliable prognostic factors that can identify patients who are at high risk of developing distant metastases despite their small tumor are needed.¹³⁻¹⁵ In previous validation studies, the 70-gene MammaPrint™ signature accurately distinguished patients with a good prognosis from those with a poor prognosis in both node-negative and node-positive breast cancer.¹⁶⁻²¹ The aim of our study was to evaluate the prognostic value of the 70-gene signature in small pT1 tumors. In addition, we investigated whether the 70-gene signature could provide clinical utility; that is, if it was able to identify a subgroup of patients with pT1ab tumors with a poor prognosis as indication for chemotherapy and a subgroup of patients with a pT1c tumor and a good prognosis as indication for no adjuvant treatment or endocrine therapy only. We merged databases from previous studies to overcome the underrepresentation of pT1 tumors.^{16-18,20-23}

Methods

Patients

For this study we selected patients with pT1 tumors from previously reported studies.^{16-18,20-23} Selection criteria for the initially reported studies are depicted in *Supplementary Figure 1*. For 2 series (*i.e.*, 295-series and RASTER-series) the follow-up was updated since initial publication (median updated follow-up 10.3 and 2.4 years, respectively).^{16,22} Each of the series were consecutive selections from the comprehensive institutional tissue banks. Patients in series 1, 4, 5, and 6 received adjuvant systemic therapy according to national guidelines applicable at that time.^{16,18,20,21,24} Patients from series 2 and 7 were selected based on adjuvant systemic therapy received, that is, no adjuvant systemic therapy for patients in series 2 and adjuvant tamoxifen monotherapy for patients in series 7.^{17,23} Patients from series 3 (prospective

RASTER trial) were treated according to the national Dutch guideline and the result of the 70-gene signature.^{22,24} There were 15 patients, all classified as poor prognosis-signature (1.6%), who received adjuvant trastuzumab. All individual studies were approved by the ethical committee of the respective hospitals.

70-gene MammaPrint™ signature

Frozen tumor samples were processed at Agendia's laboratories (Amsterdam, the Netherlands), for RNA isolation, amplification, and labeling as previously described.^{7,25} Samples were eligible for RNA isolation if they contained at least 30–50% tumor cells on hematoxylin/eosin stained sections. To assess the mRNA expression level of the 70 genes, RNA was hybridized to a custom-designed array (MammaPrint™), blinded to clinical data, at Agendia's ISO17025-certified and CLIA accredited laboratories. Tumors were classified as 70-gene good or poor prognosis signature at time of the initial series as described previously.^{16-18,20-23} On average, the 70-gene signature could be performed in 81% of the patients, which is in accordance with our previously published feasibility study.²⁶ When sufficient RNA could be extracted, the success rate of hybridization was 100%. For detailed information about dropout of patients because of tumor cell content or RNA quality, we refer to the initial publications.

Endpoints

Endpoints considered were time from surgery to distant metastasis (DMFS), and breast cancer specific survival (BCSS), defined as time from surgery to breast cancer-related death. For the analysis of distant metastasis-free survival (DMFS) we considered distant metastases as failure; patients were censored on date of death from causes other than breast cancer or date of last follow-up visit. For the analysis of BCSS, patients were censored on date of last follow-up or date of death from causes other than breast cancer. Clinicopathological data were collected as previously reported, and databases were pooled for our current study (M. Knauer, unpublished).

Statistical analyses

Associations between 70-gene signature and classical clinicopathological factors were studied using chi-square and Mann–Whitney tests. Kaplan–Meier survival analyses and log-rank tests were used to assess the difference in distant metastasis-free survival (DMFS) and breast cancer-specific survival (BCSS) of the predicted good- and poor prognosis groups by the signature. Cox proportional hazard analyses were used to calculate univariate and multivariate hazard ratios (HR) and their 95% confidence intervals (95% CI). Multivariate Cox proportional hazard analyses included the 70-gene signature and known

clinicopathological prognostic factors. We missed information about grade (n = 10), nodal status (n = 8), ER status (n = 1), and adjuvant systemic therapy (n = 4) for a small proportion of the patients. These patients were excluded for the multivariate Cox proportional hazard analyses. HRs for DMFS and BCSS at 10 years were calculated with right-censoring follow-up > 10 years, because timing of collection of follow-up data differed for the 7 series. Hazard ratios with their 95% CI for the 7 patient series were displayed on forest plots and tested for heterogeneity using a chi-square test with 6 degrees of freedom. All *p*-values are two-sided. Analyses were performed using SPSS version 15.0 (SPSS Inc, Chicago, IL) and Revman 5 (Review Manager) (www.cc-ims.net/revman).

Results

A total of 964 patients with pT1 tumors were selected from the 7 studies (*Supplementary Figure 1*).^{16-18,20-23} Among the 964 patients, 139 patients (14%) had a pT1ab tumor, 825 patients (86%) had a pT1c tumor, 693 patients (72%) had node-negative breast cancer, and 263 patients (27%) had node-positive breast cancer. During follow-up (median 7.1 years; range 0.2-25.2 years) 154 patients developed distant metastases and 155 patients died, of whom 130 of breast cancer.

The signature classified 525 tumors (54%) as good prognosis and 439 (46%) tumors as poor prognosis. A poor prognosis signature was associated with younger age at diagnosis, invasive ductal carcinoma, poorly differentiated, ER negative and HER2 positive tumors. In addition, patients with a 70-gene poor prognosis tumor more often received adjuvant systemic therapy (*Table 1*).

DMFS and BCSS were significantly better in the good prognosis group (*Figure 1A and B*). The probability of remaining free of distant metastases at 5 and 10 years were 95% (SE 1%) and 87% (SE 2%), respectively for the good prognosis-signature group, and 80% (SE 2%) and 72% (SE 3%), respectively for the poor prognosis-signature group (*Figure 1A*). A poor prognosis-signature was associated with worse DMFS at 10 years, with a univariate hazard ratio (HR) of 2.70 (95% CI 1.88-3.88; *p* < 0.001). The 5- and 10-year BCSS probabilities were 99% (SE 1%) and 91% (SE 2%), respectively, for the good prognosis group and 88% (SE 2%) and 72% (SE 3%), respectively, for the poor prognosis-signature group, with a univariate HR of 4.22 (95% CI 2.70-6.60; *p* < 0.001) at 10 years (*Figure 1B*). Forest plots of univariate hazard ratios for the signature in each individual series showed no significant heterogeneity for the prognostic value of the signature for both DMFS (chi square = 6.18; *p* = 0.4) and BCSS (chi square = 5.99; *p* = 0.4) (*Supplementary Figure 2*).

Table 1. Association between clinicopathological characteristics and the 70-gene signature.

	Good prognosis profile		Poor prognosis profile		P-value*
Surgery					0.09
BCT	402	76.6%	315	71.8%	
Mastectomy	123	23.4%	124	28.2%	
Age					<0.001
≤ 50 yrs	282	53.7%	290	66.1%	
> 50 yrs	243	46.3%	149	33.9%	
Histology					<0.001
IDC	435	82.9%	399	90.9%	
ILC	54	10.3%	17	3.9%	
Others	36	6.8%	23	5.2%	
Tumor size					0.13
pT1a/b	84	16.0%	55	12.5%	
pT1c	441	84.0%	384	87.5%	
Nodal status					0.69
Node negative	380	73.1%	313	71.8%	
Node positive	140	26.9%	123	28.2%	
Unknown	5		3		
Grade					<0.001
Grade 1	224	43.0%	56	12.9%	
Grade 2	248	47.6%	164	37.9%	
Grade 3	49	9.4%	213	19.2%	
Unknown	4		6		
Estrogen-receptor status					<0.001
Positive	513	97.7%	295	67.4%	
Negative	12	2.3%	143	32.6%	
Unknown	0		1		
HER2 status					<0.001
Negative	402	95.7%	253	77.6%	
Positive	18	4.3%	73	22.4%	
Unknown	105		113		
Adjuvant systemic therapy					<0.001
None	357	68.3%	195	44.6%	
HT only	113	21.6%	79	18.1%	
CT only	22	4.2%	76	17.4%	
HT & CT	31	5.9%	87	19.9%	
Unknown	2		2		
Total	525	100.0%	439	100.0%	

* Missing data were not used for calculation of *p*-values

BCT, breast-conserving therapy; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; HT, hormonal therapy; CT, chemotherapy.

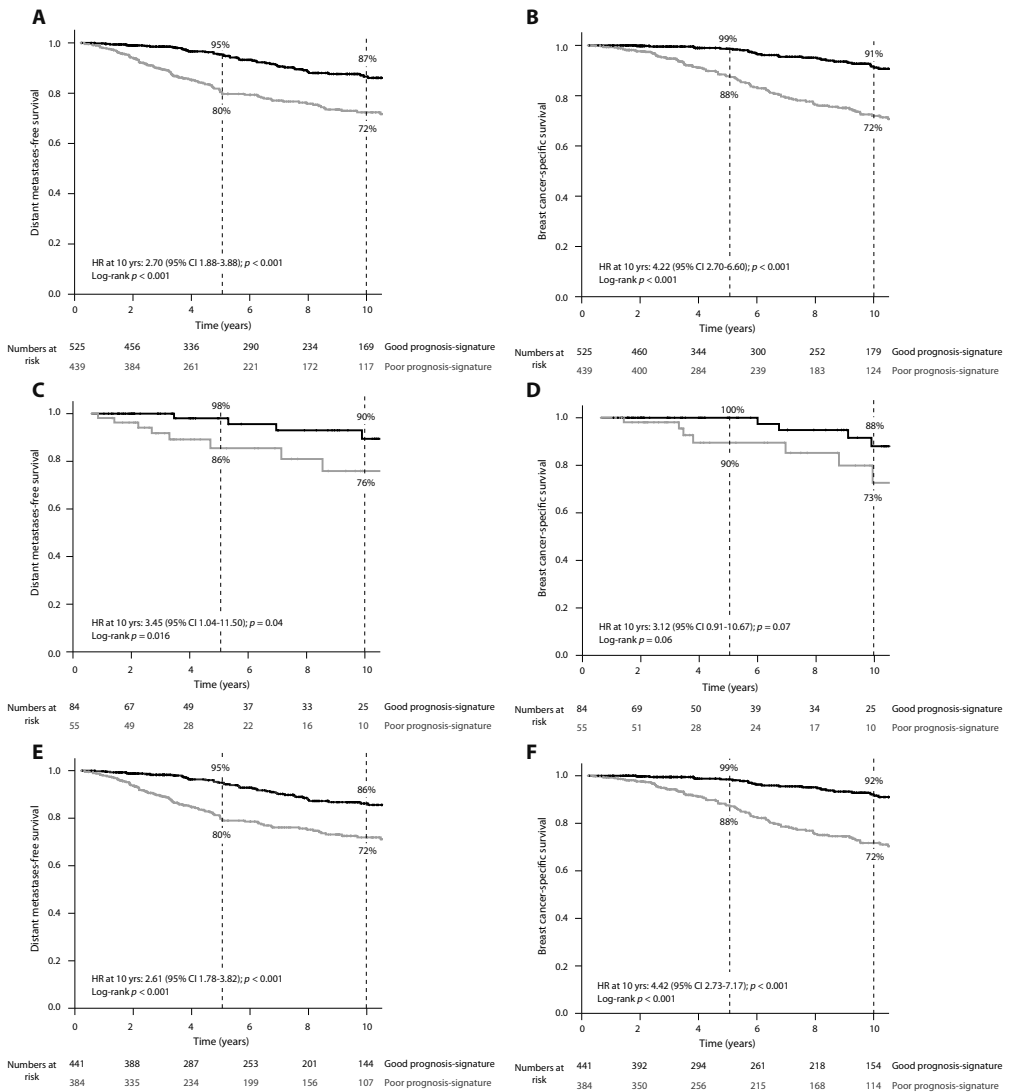


Figure 1. Kaplan-Meier curves and univariate hazard ratio (HR) for distant metastasis-free survival (DMFS) and breast cancer-specific survival (BCSS) by 70-gene prognosis-signature for 964 patients with pT1 breast tumors (A and B), for 139 patients with pT1ab tumors (C and D), and for 825 patients with pT1C tumors (E and F).

Univariate analysis showed that besides the 70-gene signature, age, histology, tumor grade, ER status, HER2 status, and type of surgery were significant predictors for DMFS at 10 years. The 70-gene signature, age, tumor grade, ER status, and HER2 status were significant predictors for BCSS at 10 years (*Supplementary table 1*). In a multivariate model, the 70-gene signature was the strongest predictor for DMFS, with an adjusted HR of 2.43 (95% CI 1.56-3.77; $p < 0.001$). In addition to the signature, nodal status and adjuvant systemic therapy were independent significant predictors for DMFS. For BCSS, again the signature, nodal status, and adjuvant chemotherapy were independent prognostic factors with adjusted HRs of 3.25 (95% CI 1.92-5.51; $p < 0.001$), 1.70 (95% CI 1.12-2.57; $p = 0.01$) and 0.41 (95% CI 0.22-0.75; $p = 0.004$), respectively (*Table 2*).

Table 2. Multivariate Cox proportional hazard analyses for distant metastasis-free survival and breast cancer-specific survival at 10 years.

Variable	Distant metastases			Breast cancer-specific survival		
	HR	CI	P-value	HR	CI	P-value
MammaPrint (poor versus good signature)	2.43	1.56-3.77	<0.001	3.25	1.92-5.51	< 0.001
Age (years)	0.99	0.97-1.01	0.31	0.98	0.96-1.01	0.16
Histology						
ILC (<i>versus</i> IDC)	1.41	0.70-2.83	0.33	1.70	0.81-3.57	0.16
Other (<i>versus</i> IDC)	0.39	0.12-1.26	0.12	0.46	0.14-1.48	0.19
Tumor size (11-20 mm versus ≤10 mm)	1.07	0.59-1.97	0.82	0.88	0.46-1.67	0.69
Nodal status (negative versus positive)	1.61	1.13-2.29	0.01	1.70	1.12-2.57	0.01
Grade						
Grade 2 (<i>versus</i> grade 1)	1.28	0.77-2.11	0.34	1.31	0.72-2.39	0.38
Grade 3 (<i>versus</i> grade 1)	1.65	0.93-2.92	0.09	1.53	0.79-2.96	0.21
ER status (negative versus positive)	0.92	0.58-1.46	0.72	1.36	0.85-2.17	0.20
HER2/NEU status						
Positive (<i>versus</i> negative)	1.19	0.69-2.04	0.53	1.48	0.84-2.61	0.18
Unknown (<i>versus</i> negative)	0.75	0.48-1.19	0.22	0.94	0.57-1.55	0.80
Surgery (mastectomy versus BCT)	1.38	0.95-1.99	0.09	1.23	0.82-1.85	0.32
Hormonal therapy (versus no hormonal therapy)	0.55	0.34-0.89	0.02	0.61	0.34-1.07	0.09
Chemotherapy (versus no chemotherapy)	0.50	0.29-0.84	0.01	0.41	0.22-0.75	0.004

Multivariate models included 941 patients due to missing values for nodal status, grade, and/or ER status in 23 patients.

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; BCT, breast-conserving therapy; HR, hazard ratio; CI 95%, confidence interval.

Besides our initial selection of patients with tumors ≤ 20 mm, we divided our study cohort into patients with pT1ab tumors (≤ 10 mm) ($n = 139$) and patients with pT1c tumors (11-20 mm) ($n = 825$). Of the patients with a pT1ab tumor, 40% were classified as having a 70-gene poor prognosis tumor. The DMFS in these 55 patients was significantly worse compared with the DMFS in patients with a good prognosis signature tumor during the entire follow-up (log rank $p = 0.016$) and at 10 years (HR 3.45 [95% CI 1.04-11.50; $p = 0.044$]). The same trend was seen for BCSS at 10 years, albeit borderline significant (Figure 1, panel C and D). The number of events was too small to calculate adjusted HRs in patients with pT1ab tumors. In patients with a pT1c tumor the 70-gene signature was a prognostic factor for both DMFS and BCSS at 10 years (HR 2.61 [95% CI 1.78-3.82; $p < 0.001$] and 4.42 [95% CI 2.73-7.17; $p < 0.001$], respectively) (Figure 1, panel E and F).

Among the 964 patients in our study cohort, 552 patients (57%) received no adjuvant systemic therapy, 408 patients (42%) received endocrine- and/or chemotherapy and for 4 patients (1%) adjuvant systemic therapy was unknown. The 70-gene signature retained its prognostic value in adjuvantly untreated patients, with adjusted HRs of 2.54 (95% CI 1.49-4.34; $p = 0.001$) and 3.47 (95% CI 1.83-6.60; $p < 0.001$) for DMFS and BCSS, respectively (Supplementary Figure 3). In addition, the 70-gene MammaPrint™ signature was an independent prognostic factor in 788 patients with ER positive tumors for both DMFS and BCSS, with adjusted HRs of 2.51 (95% CI 1.60-3.95; $p < 0.001$) and 3.43 (95% CI 1.98-5.95; $p < 0.001$), respectively.

Discussion

Our study showed that the 70-gene MammaPrint™ signature that has been validated as an independent prognostic factor in node-negative and node-positive breast cancer is also an independent prognostic factor in patients with small breast tumors. The signature accurately distinguished patients with a good outcome from those with a poor outcome in our study cohort of patients with pT1 tumors. Interestingly, our results showed that even a considerable proportion of small tumors have a substantial metastatic capacity, which can be identified by the 70-gene signature (28% distant relapse rate at 10 years in tumors classified as poor prognosis by the signature). Therefore, the 70-gene signature can be of use in daily clinical practice to optimize and individualize treatment decision-making in this growing breast cancer population of patients with pT1 tumors.

Historical tumor banks contain particularly large tumors, which is in contrast to the actual increase in proportion of small tumors diagnosed, due to mammographic screening. As a consequence, most studies of potential prognostic markers to date included fewer than 10% of tumors smaller than 10 mm.^{7,27-29} To overcome this potential problem of underrepresentation of small tumors in repositories we selected patients with pT1 tumors from previous studies. One of the potential limitations of our pooled database is therefore

the heterogeneity of our study population, especially with regard to years of diagnosis and adjuvant systemic therapy. However, there was no evidence of significant heterogeneity among the various series that provided pT1 cases for our study, for both DMFS and BCSS (*Supplementary Figure 2*). Another potential bias with regard to adjuvant systemic therapy in our study cohort is the fact that patients from the more recent prospective RASTER trial were partially treated based on the outcome of the 70-gene signature.²² However, when we excluded these patients from our series (n = 301) the 70-gene prognosis signature retained its independent prognostic value with adjusted HRs for DMFS and BCSS of 2.32 (95% CI 1.48-3.63; $p < 0.001$) and 3.09 (95% CI 1.82-5.24; $p < 0.001$), respectively. Patients with pT1 tumors selected from series 1 included 31 patients whose data were used in the development of the 70-gene signature, thereby potentially causing an overestimation of the prognostic value of the signature.⁷ Excluding these patients from our analyses resulted in similar adjusted HR for both DMFS and BCSS at 10 years (2.24 [1.41-3.54; $p = 0.001$] and 3.10 [1.79-5.37; $p < 0.001$] respectively).

Our study cohort contained 139 patients with pT1ab tumors, and in this small group of patients with pT1ab tumors 15 distant metastases (DM) and 11 breast cancer-specific deaths (BCSD) occurred. The 70-gene signature was able to distinguish patients with pT1ab tumors who developed DM from those who did not (log rank $p = 0.016$ and HR 3.45, $p = 0.044$). For BCSS the same trend was observed, but did not, however, reach statistical significance, which is most likely because of the low number of events in combination with the relatively small patient population with pT1ab tumors. The results of our study suggest that the 70-gene signature can select patients with pT1ab tumors with a higher risk of developing DM (24% at 10 years), who thus might be candidates for adjuvant systemic therapy. In addition, the signature can identify patients with a pT1c tumor with a relatively low risk of developing DM (14% at 10 years), who might be sufficiently treated with endocrine therapy, as the large majority (98%) is ER positive. Since adjuvant systemic treatment recommendation for patients with small tumors is a matter of debate, our results provide evidence that selecting patients with pT1 tumors using the 70-gene signature could be relevant for adjuvant systemic therapy recommendation. For patients with pT1ab tumors the data suggest the same, though we will have to await results of further studies. With the current development of RNA extraction from FNA and core biopsies for microarray gene expression analyses, gene expression profiles will become available to a larger extent for patients with very small tumors.³⁰

Hanrahan and colleagues showed that, in addition to a relatively wide range of observed relapse-free survival rates in patients with pT1abN0 tumors, histological grade, ER negative tumor, younger age at diagnosis (<50 years), lymphovascular invasion (LVI), high Ki-67, HER2/NEU positivity, and larger tumor size were associated with poor outcome.^{8,9} In our database of patients with pT1 tumors, we confirmed the univariate prognostic value of age, tumor grade, ER and HER2/NEU (*Supplementary Table 1*). Among the 954 patients with known tumor grade, 692 patients had a grade 1 or 2 tumor and 262 patients had a grade 3 tumor.

Patients with a grade 1–2 tumor had a significantly lower 10 -year distant relapse rate compared with those with a grade 3 tumor (16 and 29%, respectively), with a univariate HR of 2.24 (95% CI 1.54–3.08; $p < 0.001$) (*Supplementary Figure 4*). However, the event rate in patients considered to have a ‘good prognosis’ based on grade (*i.e.*, with grade 1–2 tumors) was relatively large. Specifically, classification based on grade (*i.e.*, grade 1–2 considered low risk) resulted in misclassification of 43 additional DM and 41 additional BCSD, compared with 4 additional misclassified DM and 3 BCSD when classified by the 70-gene signature. Classification based on ER status, would result in a large ‘good-prognosis’ group (*i.e.*, 84% is ER positive), with a relatively high event rate in this group (19% DM at 10 years) (data not shown). Therefore, ER status alone would not be useful to select patients with pT1 tumors for adjuvant systemic chemotherapy. These results show that while the proportion of patients classified as having a ‘good prognosis’ by both grade and ER status increased compared with the good prognosis group by the 70-gene signature, the prediction of outcome becomes less accurate and an increased proportion of events were missed. Moreover, results of multivariate analyses showed that the prognostic information that is captured by ER and grade is not independent of other factors. In fact, only the 70-gene signature, nodal status, and adjuvant systemic therapy were independent prognostic factors for DMFS and BCSS in this study cohort.

Tumors identified as 70-gene signature low risk and grade 1/2 showed a considerable proportion of events (51 DM and 32 BCSD, respectively). However, these misclassified events occurred significantly later compared with the accurately classified events, 7.4 years (SE 0.4) *versus* 3.1 years (SE 0.2), respectively, for DM ($p < 0.001$) and 9.3 yrs (SE 0.5) *versus* 4.6 years (SE 0.3), respectively, for BCSD ($p < 0.001$). Previous studies have already shown a time dependency for the prognostic value of the 70-gene signature, and our results support once more the hypothesis of a different biological mechanism for early and late relapses.^{17,21} Moreover, our results show the still unmet need for markers to predict late events. The accuracy of the 70-gene MammaPrint™ signature in predicting early events coincides with the effect of chemotherapy, as that is known to be most beneficial in the first 5–7 years after diagnosis and would thus potentially prevent the occurrence of early metastasis in the poor prognosis group.³¹

As a consequence of adjuvant treatment guidelines, a substantial proportion of patients in this validation series (216 of 964 patients, 22%) received adjuvant chemotherapy, with or without hormonal therapy. Patients classified as poor prognosis by the 70-gene signature more often received adjuvant chemotherapy (37 *versus* 10% in the good prognosis group, respectively; $p < 0.001$). Tumor characteristics in the poor signature group, that is, more ER-negative and poorly differentiated, are generally believed to be associated with a higher likelihood of response to chemotherapy.³¹ Moreover, Bender and colleagues recently showed that the benefit of chemotherapy was exclusively seen in patients classified as poor prognosis by the 70-gene signature.³² This larger efficacy of chemotherapy in combination with the larger proportion of chemotherapy-treated patients in the poor prognosis

signature group would imply that the prognostic value of the 70-gene signature as shown in our series is underestimated and would potentially be higher in an untreated group. In conclusion, our study shows that the 70-gene signature is a strong and independent prognostic factor for patients with pT1 tumors. In addition, we show that a considerable proportion of small tumors has metastatic potential, supporting the idea that metastatic capacity is an early genetic inheritance that can be revealed by the 70-gene signature. Consequently, selecting patients with pT1 tumors based on the signature will result in a more accurate allocation of adjuvant systemic therapy in this patient population.

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Conflicts of Interest

Laura J Van 't Veer is named inventor on a 70 gene prognosis-signature patent. Laura J Van 't Veer reports holding equity in Agendia BV.

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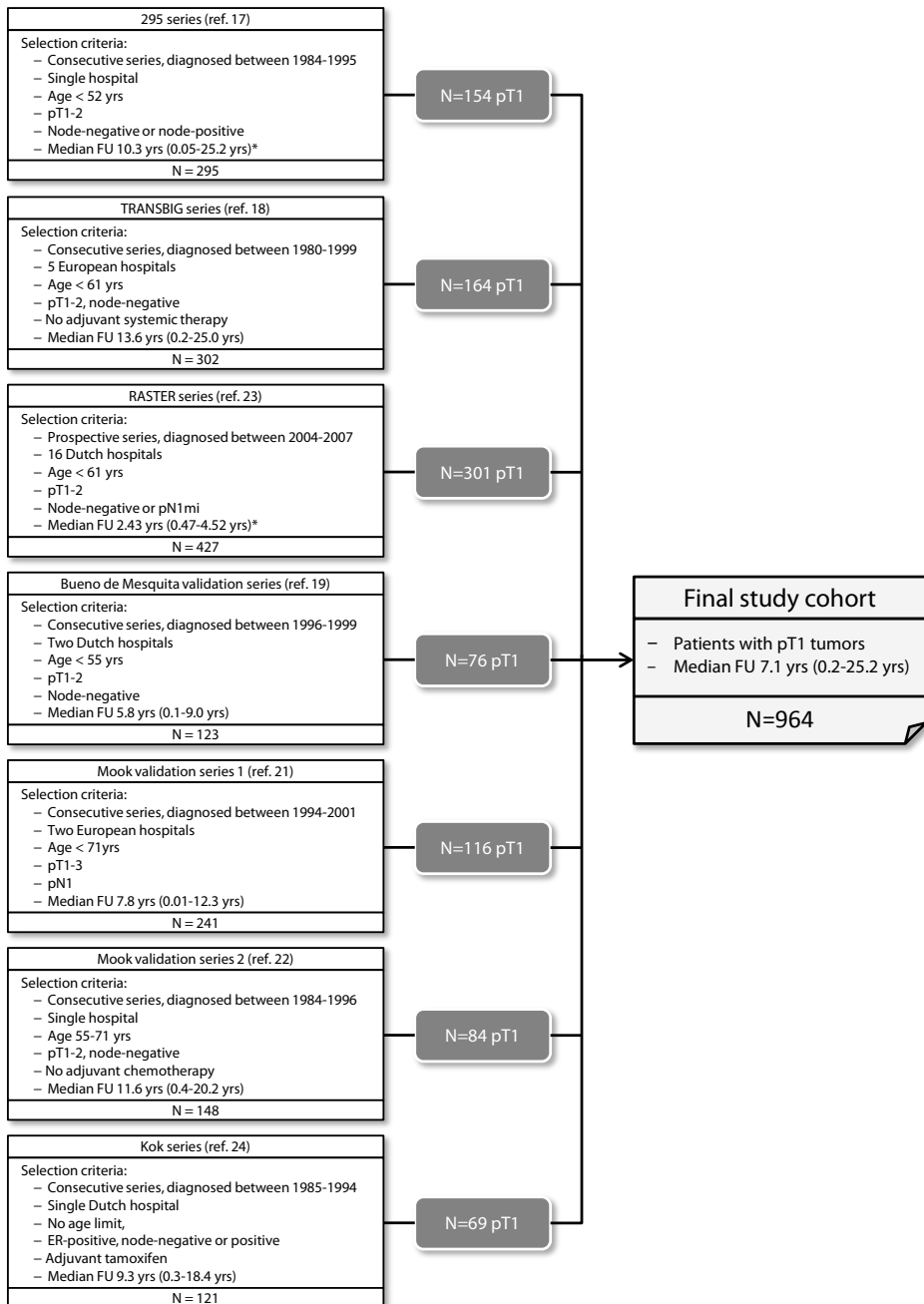
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Supplements Chapter 6

Supplementary Table 1. Univariate Cox-regression analyses for distant metastasis-free survival and breast cancer-specific survival at 10 years.

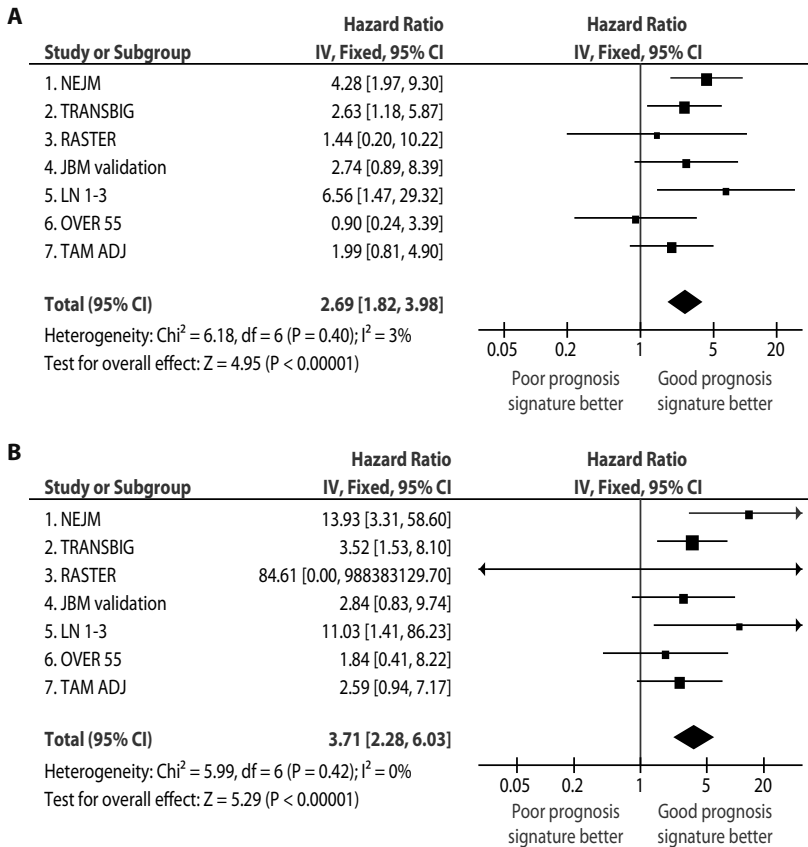
Variable	Distant metastases			Breast cancer-specific survival		
	HR	CI	P-value	HR	CI	P-value
MammaPrint (poor <i>versus</i> good signature)	2.70	1.88-3.88	<0.001	4.22	2.70-6.60	<0.001
Age (years)	0.98	0.96-0.996	0.02	0.97	0.95-0.99	0.004
Histology						
ILC (<i>versus</i> IDC)	0.96	0.49-1.90	0.91	1.07	0.52-2.21	0.85
Other (<i>versus</i> IDC)	0.37	0.09-0.88	0.05	0.45	0.17-1.23	0.12
Tumor size (11-20 mm <i>versus</i> ≤10 mm)	1.49	0.82-2.69	0.19	1.23	0.66-2.29	0.52
Nodal status (negative <i>versus</i> positive)	1.07	0.94-1.21	0.29	0.94	0.77-1.16	0.58
Grade						
Grade 2 (<i>versus</i> grade 1)	1.57	0.97-2.54	0.07	1.85	1.04-3.29	0.04
Grade 3 (<i>versus</i> grade 1)	2.89	1.79-4.67	<0.001	3.82	2.18-6.71	<0.001
ER status (negative <i>versus</i> positive)	1.78	1.19-2.65	0.005	2.86	1.91-4.29	<0.001
HER2/NEU status						
Positive (<i>versus</i> negative)	1.67	1.00-2.78	0.05	2.43	1.42-4.14	0.001
Unknown (<i>versus</i> negative)	0.89	0.60-1.32	0.56	1.22	0.80-1.87	0.35
Surgery (mastectomy <i>versus</i> BCT)	1.54	1.09-2.20	0.02	1.43	0.96-2.11	0.08
Hormonal therapy (<i>versus</i> no hormonal therapy)	0.73	0.49-1.08	0.11	0.67	0.42-1.05	0.08
Chemotherapy (<i>versus</i> no chemotherapy)	1.04	0.68-1.58	0.88	0.98	0.60-1.59	0.93

IDC, Invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; BCT, breast-conserving therapy; HR, hazard ratio; CI, 95% confidence interval.



Supplementary figure 1. Patient selection.

* Updated follow-up (FU).

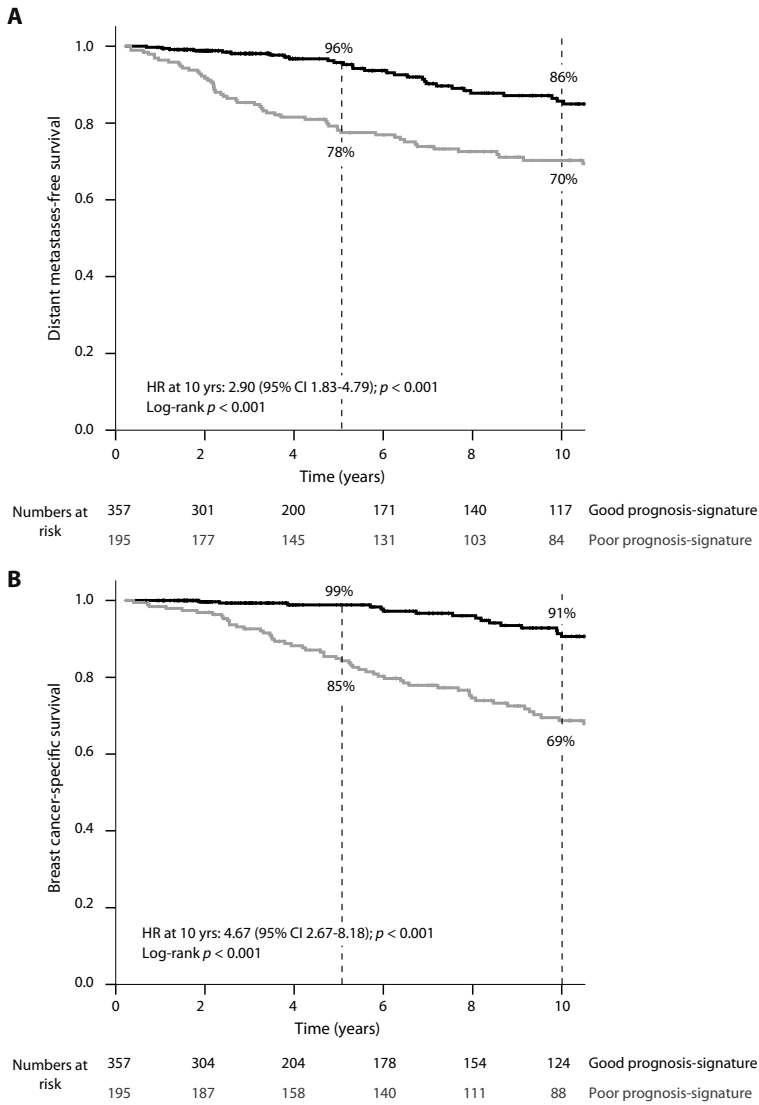


Supplementary figure 2. Forest plots of hazard ratios (HRs) and 95% confidence intervals (95% CI) for each series in the poor prognosis *versus* good prognosis group by the 70-gene signature. Squares = HRs; Lines = 95% CIs; Diamond = weighted total HR and 95% CI.

A. Distant metastasis-free survival at 10 years.

B. Breast cancer-specific survival at 10 years.

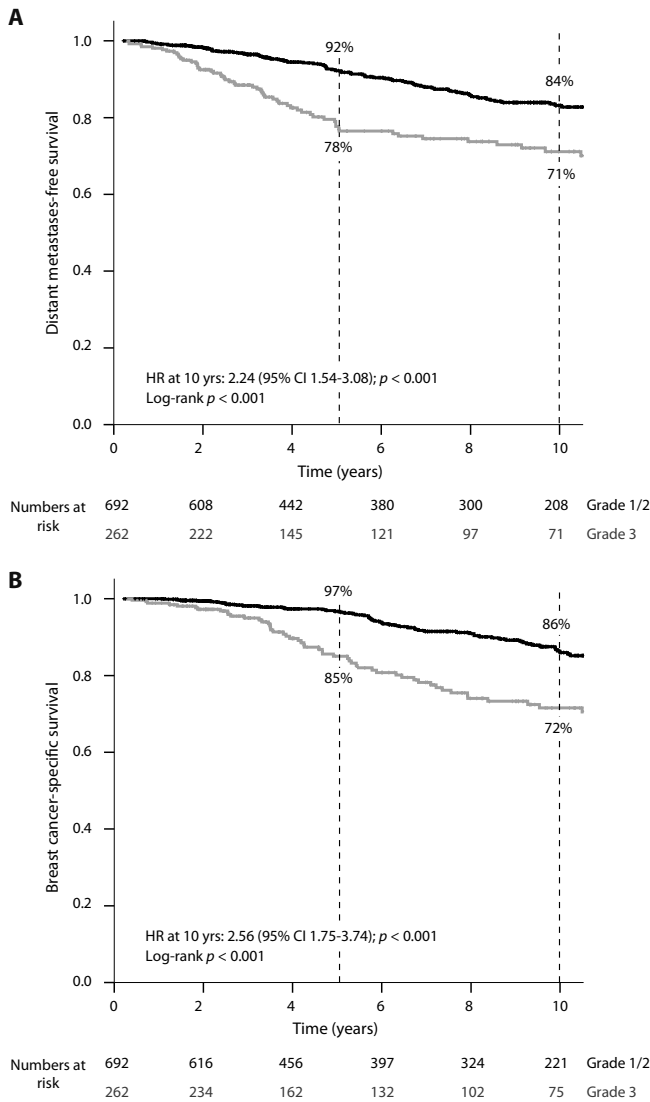
NEJM = 295 series¹⁷; TRANSBIG = TRANSBIG series¹⁸; RASTER = RASTER series²³; JBM validation = Bueno de Mesquita validation series¹⁹; LN 1-3 = Mook validation series 1²¹; OVER 55 = Mook validation series 2²²; TAM ADJ = Kok series²⁴.



Supplementary figure 3. Kaplan-Meier curves and univariate hazard ratio (HR) by 70-gene prognosis-signature for patients who did not receive adjuvant systemic therapy (n=552).

A. Distant metastasis-free survival.

B. Breast cancer-specific survival.



Supplementary figure 4. Kaplan-Meier curves and univariate hazard ratio (HR) by grade 1/2 versus grade 3 (n=954, tumor grade was missing for 10 patients).

A. Distant metastasis-free survival.

B. Breast cancer-specific survival.