



UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Prognostic factors in breast cancer: one fits all?

Mook, S.

Publication date
2011

[Link to publication](#)

Citation for published version (APA):

Mook, S. (2011). *Prognostic factors in breast cancer: one fits all?* [Thesis, fully internal, Universiteit van Amsterdam].

General rights

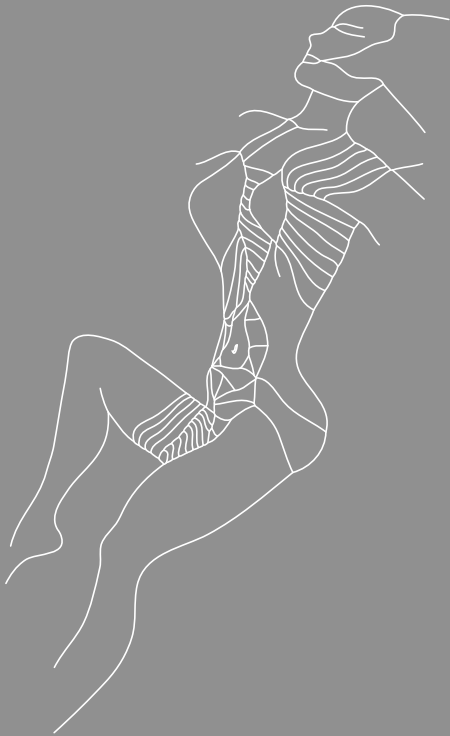
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

Chapter 7

The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer



Michael Knauer
Stella Mook
Emiel J.T. Rutgers
Richard A. Bender
Michael Hauptmann
Marc J. Van de Vijver
Rutger H. Koornstra
Jolien M. Bueno de Mesquita
Sabine C. Linn
Laura J. Van 't Veer

Abstract

Multigene assays have been developed and validated to determine the prognosis of breast cancer. In this study, we assessed the additional predictive value of the 70-gene MammaPrint™ signature for chemotherapy (CT) benefit in addition to endocrine therapy (ET) from pooled study series. For 541 patients who received either ET (n = 315) or ET + CT (n = 226), breast cancer-specific survival (BCSS) and distant disease-free survival (DDFS) at 5 years were assessed separately for the 70-gene high and low risk groups. The 70-gene signature classified 252 patients (47%) as low risk and 289 (53%) as high risk. Within the 70-gene low risk group, BCSS was 97% for the ET group and 99% for the ET + CT group at 5 years with a non-significant univariate hazard ratio (HR) of 0.58 (95% CI 0.07–4.98; $P = 0.62$). In the 70-gene high risk group, BCSS was 81% (ET group) and 94% (ET + CT group) at 5 years with a significant HR of 0.21 (95% CI 0.07–0.59; $P < 0.01$). DDFS was 93% (ET) *versus* 99% (ET + CT), respectively, in the 70-gene low risk group, HR 0.26 (95% CI 0.03–2.02; $P = 0.20$). In the high risk group DDFS was 76 *versus* 88%, HR of 0.35 (95% CI 0.17–0.71; $P < 0.01$). Results were similar in multivariate analysis, showing significant survival benefit by adding CT in the 70-gene high risk group. A significant and clinically meaningful benefit was observed by adding chemotherapy to endocrine treatment in 70-gene high risk patients. This benefit was not significant in low risk patients, who were at such low risk for recurrence and cancer-related death, that adding CT does not appear to be clinically meaningful.

Introduction

Adjuvant treatment for early stage breast cancer has significantly improved patient outcome in recent years. In addition to population based screening programs enabling early detection, the increase in the use of endocrine treatment and chemotherapy (CT) has led to a decrease in breast cancer mortality in many countries over the past decade.¹ Indications for adjuvant therapy differ among several treatment guidelines, including the National Comprehensive Cancer Network (NCCN) and St. Gallen consensus conference guidelines.^{2,3} The prevailing paradigm is that adjuvant CT and endocrine therapy (ET) may rather be additive and are known not to be of comparable reduction in the odds of recurrence across all risk groups.¹

In a web-based survey among leading breast cancer specialists in 2007, the identification of molecular signatures for better selection of CT treatment benefit was voted as the most important priority for breast cancer research.⁴ Several multigene assays have been developed within the last few years to identify patients with a high or low risk of recurrence and death from breast cancer. Four of these multigene predictors have been validated in independent patient series and are now commercially available: they include the 70-gene signature (MammaPrint™),^{5,6} the 21-gene recurrence score (RS) (Oncotype DX),^{7,8} the genomic grade index (MapQuant Dx)⁹ and the HOXB13/IL17R, and molecular grade index (Theros Breast Cancer Index).^{10,11} Although most experts now agree on the prognostic value of these assays, evidence regarding their predictive value (*i.e.*, the degree of benefit from (neo)adjuvant CT for the different risk groups) is limited.^{3,12}

The 70-gene signature, which is being performed on fresh (frozen) tumor tissue, has shown to have a strong prognostic power for early breast cancer patients.^{5,6,13,14} The 70-gene set was developed using the so-called data-driven approach, *i.e.*, without a priori knowledge of the role of the involved genes, whereas for example the 21-gene RS used the knowledge-driven approach, *i.e.*, genes known to be involved in breast cancer formation and metastatic spreading were included. In this study, we investigated whether patients with a low risk 70-gene signature are at sufficiently low risk of distant metastasis and death, that the addition of CT to endocrine treatment alone provides little or no benefit, whereas patients in the high risk group show a significant and clinically relevant benefit of adding CT to endocrine treatment.

Patients and methods

Patients

A pooled database from seven previously reported studies, including 1637 patients with known adjuvant treatment status (1637/1696, 97%) was developed. Patients who met the following criteria were selected: unilateral stage pT1-3, N0-1, M0 invasive breast carcinoma diagnosed between 1984 and 2006, surgical treatment with either breast-conserving therapy or mastectomy with sentinel node biopsy or axillary lymph node dissection followed by radiotherapy, if indicated.¹⁵ For this analysis, disease was staged according to the 2002 UICC TNM-classification, 6th edition. All involved studies had been approved by the respective institutional review boards. We evaluated all patients who had received either ET alone or ET plus adjuvant CT (ET + CT). In the whole patient population, 90% of patients were estrogen receptor (ER) positive and 69% of the study patients were progesterone receptor (PR) positive. The studies by Van de Vijver *et al.*,⁶ Bueno de Mesquita *et al.*,^{14,16} Mook *et al.*,^{17,18} and Kok *et al.* (personal communication) were included, resulting in the inclusion of 30, 182, 29, 154, 27 and 119 patients from the database, respectively. Differences in adjuvant CT benefit (CMF or anthracycline +/- taxane regimens) within the 70-gene low risk and high risk patients were assessed. Of 226 patients treated with adjuvant CT, 11 patients received CMF, 21 patients received taxane containing regimens, and the vast majority of 194 patients received different anthracycline-containing regimens. Time-to-event analyses using updated and centrally verified individual patient data were performed using a pooled database (Microsoft Access; Microsoft, Redmond, WA).

Microarray analysis

Frozen tumor samples from each patient were processed at Agendia's laboratory (Amsterdam, The Netherlands), for RNA isolation, amplification, and labeling as previously described.^{5,19} Samples were eligible for RNA isolation, if they contained at least 30% 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™) at Agendia's ISO17025-certified, CLIA accredited, and FDA-cleared laboratory. Tumors were classified as having a 70-gene high or low risk-signature at the time of initial series as described previously, and were blinded to clinical data.

Statistical analyses

The endpoints evaluated were breast cancer-specific survival (BCSS), defined as time from surgery to breast cancer-related death and distant disease-free survival (DDFS), defined as time from surgery to any distant metastasis. For both outcomes, follow-up was censored at 5 years, because firstly, most of the treatment effect of adjuvant CT is observed within 5 years and secondly to control for differences in median follow-up of the included studies. Kaplan–Meier survival plots and log-rank tests were used to assess differences in BCSS and DDFS for the 70-gene profile low and high risk groups. All *P*-values were two-sided and considered statistically significant if less than 0.05. Adjusted uni- and multivariate hazard ratios (HRs) and corresponding 95% confidence intervals (95% CIs) were derived from Cox proportional hazards models. Co-variables used in adjusted models included age at diagnosis, tumor size, number of positive lymph nodes, histological grade, ER and PR status, hormonal therapy, and CT. Relative differences between treatment effects by 70-gene risk groups were assessed by adding an interaction term to the model. All statistical analyses were performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL) and SAS 9.1 (SAS Institute Inc., Cary, NC).

Results

Five-hundred-forty-one patients with 0–3 positive lymph nodes from the pooled database were either treated with ET only or endocrine plus CT and, thus, met the inclusion criteria for this study. The median follow-up for the study population was 7.1 years (range 0.1–25.2). At 5 years of follow-up, 52 patients had developed distant metastases and 33 patients had died of their disease. The 70-gene MammaPrint™ signature classified 252 patients (47%) as low risk and 289 (53%) as high risk. Detailed patient characteristics are shown in *Table 1*.

Prognostic value of the 70-gene signature

BCSS and DDFS were significantly better in the 70-gene signature low risk group. The 5-year BCSS probabilities were 97% for the low risk group and 87% for the high risk-signature group, with a univariate HR of 4.81 (95% CI 1.98–11.67; *P* < 0.01). The probability of remaining free of distant metastases at 5 years was 95% for the low risk-signature group and 82% for the high risk-signature group with a univariate HR of 3.88 (95% CI 1.99–7.58; *P* < 0.01).

Table 1. Summary of clinico-pathological characteristics of the study population.

Patients (n = 541)	Characteristics	n (%)
Age	≤ 50 years	231 (43%)
	> 50 years	310 (57%)
Tumor size	T1	279 (52%)
	T2	254 (47%)
	T3	7 (1%)
	n.a.	1 (0.2%)
Lymph node status	N0	265 (49%)
	N1	276 (51%)
Histological grade	Grade 1	134 (25%)
	Grade 2	233 (43%)
	Grade 3	163 (30%)
	n.a.	11 (2%)
Estrogen receptor status	Positive (≥ 10%)	484 (90%)
Progesterone receptor status	Positive (≥ 10%)	371 (69%)
Her2-status	Positive	59 (11%)
Adjuvant treatment	ET only	315 (58%)
	ET + CT	226 (42%)
70-gene MammaPrint signature	Low risk	252 (47%)
	High risk	289 (53%)

Abbreviations: n, number; n.a., not available; ET, endocrine therapy; CT, chemotherapy.

Adjuvant CT benefit for the 70-gene signature risk groups

In order to determine the predictive utility of the 70-gene signature, we assessed differences in survival between patients who received either ET alone or ET combined with CT, separately within the 70-gene low risk and 70-gene high risk patient groups. Univariate analysis demonstrated a significantly longer DDFS and BCSS in the 70-gene high risk group for the patients receiving both CT and endocrine treatment, whereas such a significant difference was not observed for the 70-gene low risk group. BCSS for the 70-gene low risk group was 97% for the ET group and 99% for the ET + CT group, with a univariate HR of 0.58 (95% CI 0.07–4.98; $P = 0.62$). In the 70-gene high risk group, 5-year BCSS was 81% for the ET group and 94% for the ET + CT group with a HR of 0.21 (95% CI 0.07–0.59, $P < 0.01$). The corresponding Kaplan–Meier survival curves are shown in *Figure 1A* (BCSS) and for DDFS in *Figure 1B*. In the 70-gene low risk group, DDFS probabilities at 5-years for the ET and the

ET + CT groups were 93 *versus* 99%, respectively, with a HR of 0.26 (95% CI 0.03–2.02; *P* = 0.20). In the high risk group, survival was 76 *versus* 88% for the ET and the ET + CT groups, respectively, with a HR of 0.35 (95% CI 0.17–0.71; *P* < 0.01).

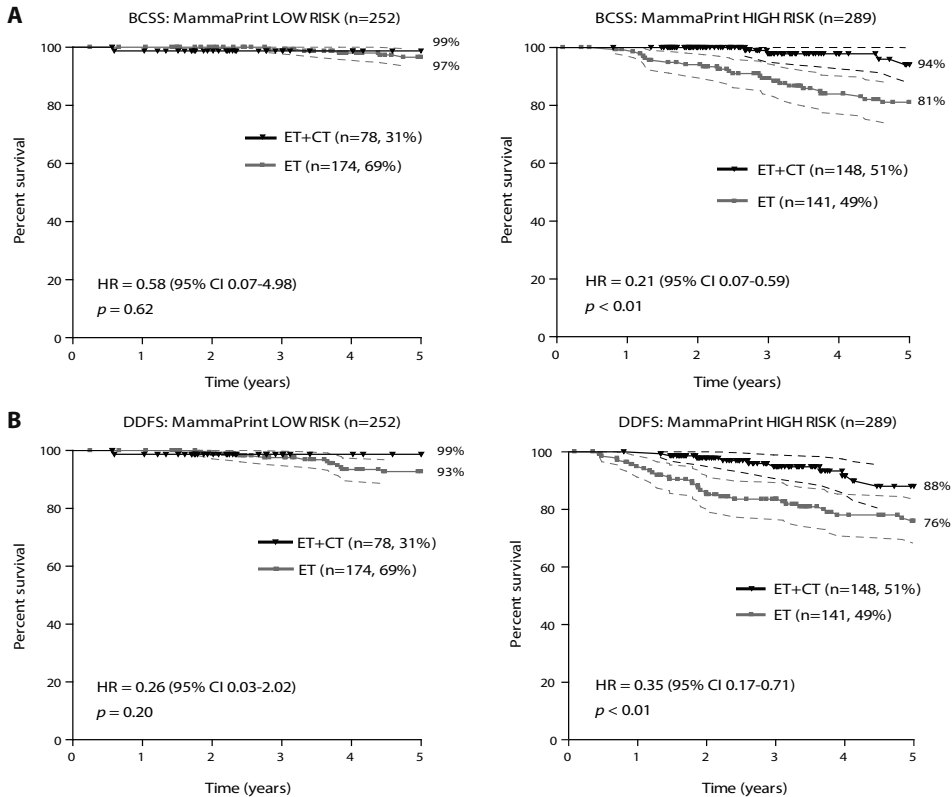


Figure 1. A. Five-year breast cancer-specific survival by treatment within the 70-gene signature groups (70-gene low risk on the left, high risk on the right).

B. Five-year distant disease-free survival by treatment within the 70-gene signature groups (70-gene low risk on the left, high risk on the right).

Abbreviations: BCSS, breast cancer-specific survival; DDFS, distant disease-free survival; n, number; ET, endocrine therapy; ET + CT, endocrine + chemotherapy; HR, univariate hazard ratio.

To further evaluate treatment effects, we compared relative and absolute differences in survival between patient groups receiving ET or ET + CT for both 70-gene risk groups. The relative differences as determined by the interaction analysis resulted in a *P*-value of 0.45.

In absolute numbers, the addition of CT for patients in the 70-gene low risk group could prevent 3 events per 1000 patient years, resulting in a number needed to treat (NNT) of 333 (95% CI 78 harm to 83 benefit, *i.e.*, there is similar chance that adding CT may result in benefit or harm for this patient group). Adding CT for patients in the 70-gene high risk group could prevent 33 events per 1000 patient years, resulting in a NNT of 30 (95% CI 19 benefit to 64 benefit).

In multivariate Cox regression analysis adjusted for age, tumor size, number of positive lymph nodes, grade, ER and PR status, and HER2-expression, the results were similar to the univariate results, indicating significant benefit in survival for adding CT in the high risk group ($P = 0.02$). Details of the multivariate analysis for BCSS are shown separately for the 70-gene high risk and low risk patient groups in *Table 2*.

Table 2. Multivariate analysis of treatment effects for several prognostic factors. BCSS for the 70-gene high risk patients is shown above and for the 70-gene low risk patients below.

MammaPrint	HR (95% CI)	P-value
High risk		
Age at diagnosis (by year)	0.96 (0.91–1.02)	0.17
Tumor size (by cm)	1.05 (1.01–1.09)	0.02
No. of positive nodes (0-3)	1.39 (0.95–2.03)	0.09
Grade	1.03 (0.48–2.19)	0.94
ER-positive status	0.48 (0.18–1.34)	0.16
PR-positive status	0.31 (0.09–1.03)	0.06
HER2-positive status	0.72 (0.25–2.10)	0.55
Adjuvant therapy: ET <i>versus</i> ET + CT	0.21 (0.06–0.80)	0.02
Low risk		
Age at diagnosis (by year)	1.00 (0.88–1.15)	0.95
Tumor size (by cm)	0.98 (0.89–1.10)	0.77
No. of positive nodes (0-3)	1.09 (0.37–3.16)	0.88
Grade	0.57 (0.12–2.82)	0.49
ER-positive status	∞ (0–∞)	0.99
PR-positive status	0.09 (0.01–0.90)	0.04
HER2-positive status	∞ (0–∞)	0.99
Adjuvant therapy: ET <i>versus</i> ET + CT	∞ (0–∞)	0.98

Abbreviations: BCSS, breast cancer-specific survival; HR, hazard ratio; 95% CI, 95% confidence interval; cm, centimeter; no, number; ER, estrogen receptor; PR, progesterone receptor; ET, endocrine therapy; CT, chemotherapy.

Discussion

This is the first study assessing the prediction of adjuvant CT benefit using the 70-gene MammaPrint™ signature in a pooled analysis of lymph node negative and positive patients. When grouped by chemo-ET or ET alone, patients in the 70-gene low risk group derive no significant survival benefit from CT added to ET. Of note, very few events were observed in this 70-gene low risk patient group, irrespective of type of adjuvant treatment, confirming their overall good outcome. Indeed, for these patients, a low gene expression result may indicate a sufficiently low risk of recurrence and cancer-related death at 5 years to obviate any benefit of adjuvant CT. In contrast, a significant and clinically meaningful benefit of combined chemo-ET was shown for the 70-gene high risk group. These observed differences in benefit for the 70-gene low and high risk group were not significant for the interaction test, comparing the differential in the extent of the benefit between 70-gene low and high risk patients. Ioannidis *et al.* have previously indicated that an interaction is not necessarily required for a predictive score to be useful in therapeutic decisions, especially when absolute risk in the low risk group is so low that CT would not be recommended.²⁰ The results from a pooled analysis of individual patient data not only confirm the 70-gene signature as a validated, independent prognostic tool, but also suggest the assay to be a predictive tool for the expected benefit of adjuvant CT in patients with early breast cancer and a high risk 70-gene profile.

One of the strengths of this study is its design using a pooled analysis of centrally reviewed and updated individual patient data, representing a commonly accepted method of a meta-analysis.²¹ While this study was not done using retrospective analysis of phase III clinical trial data, the patients studied represent an unselected early breast cancer cohort, which can be seen in *Table 1*. Moreover, the included consecutive series were obtained from prospectively collected frozen banked tumor material at several leading European cancer centers. All patients with a cancer diagnosis were accessioned into the tumor banks consecutively as they presented to the respective institutions. Clinical and pathological data shown in *Table 1* were centrally reviewed and blinded to the microarray analysis. One of the clear limitations of this study next to limited patient numbers and differences in CT regimens is its retrospective design. However, it will be several years before survival data from ongoing randomized controlled trials such as the MINDACT or TAILORx study^{22,23} will be available.

The use of multigene assays such as the 70-gene profile and the 21-gene RS has increased in recent years and these assays have impacted treatment decisions. In multiple validation studies it has been demonstrated that the 70-gene signature adds independent prognostic information to routine clinico-pathologic risk assessment.^{13,14,17} In a study of 427 breast cancer patients from 16 community-based Dutch hospitals,¹⁶ discordances in risk stratification between the 70-gene signature and treatment guidelines were noted in up to 41% of patients. This led to an adjustment of the adjuvant treatment regimen in two-third of the study cohort.

Two studies have evaluated the predictive utility of the 21-gene RS with respect to CT benefit. The advantage of this assay is its validation on tumor tissue from phase III trials with uniform CT regimens, although only a subset was available for analysis. Within the NSABP B20 study, the degree of benefit from adjuvant CT ranged from little in the low and intermediate RS to 20% absolute benefit in the highest RS group.¹² Of note, the control arm of this study had been used for development of the 21-gene RS which may have resulted in overinterpretation of the data.^{7,20} Ioannidis mentioned in his commentary that the poor performance of the RS in the CT arm of NSABP B20 caused the significant treatment-RS interaction effect. The second predictive study was done on samples from the Southwest Oncology Group study S8814 (INT0100) in node positive patients and presented at the 2007 San Antonio Breast Cancer Symposium.²⁴ No benefit in disease-free survival for the patients with a low RS for added CAF CT concurrent with tamoxifen was shown, whereas the benefit was significant in the highest RS group.

In the neoadjuvant setting a number of studies using several drugs demonstrated the predictive value of several gene signatures for CT response. These signatures comprise known signatures such as the genomic grade index as well as several new classifiers.²⁵⁻³⁷ Additional data to support the predictive potential for the 70-gene assay comes from the neoadjuvant study of Straver *et al.*³⁸ In this study, only patients who had high risk profiles were likely to achieve a pathologic complete response (pCR) to CMF or anthracycline-containing CT regimens. In fact, no patient with a low risk profile achieved a pCR and only two patients (9%) of this group achieved a partial response to therapy compared to 37% overall response ($P = 0.008$) in the high risk group including a 20% pCR rate ($P = 0.015$). The results of all these studies support the theory that gene expression profiles can separate CT-responsive from poorly or non-responsive tumors.

Clinical implications

In about two-third of all hormone receptor-positive cases, clinical and genomic risk assessment using the 70-gene signature will be concordant. If both methods indicate a high risk of recurrence, the use of combined chemo-ET seems clinically indicated. If both methods indicate a low risk of recurrence, then ET alone should be adequate treatment. For the one-third of patients with discordant risk assessment, our findings suggest consideration of the following approach. If the 70-gene profile indicates a low risk in a clinically stratified high risk patient, ET alone may be indicated in highly endocrine-responsive patients, as defined by the St. Gallen consensus panel, as these patients are at very low risk to recur and will likely gain little or no benefit from additional CT. Conversely, 70-gene high risk and clinically assessed low risk patients will likely benefit from combined chemo-endocrine treatment. If these patients are highly endocrine-responsive, then endocrine treatment alone might be the prudent option, however, withholding adjuvant therapy might not be a prudent option for this group of patients. Furthermore, other

factors such as age and co-morbidities may influence shared decision-making for adjuvant systemic therapy. However, generally definitive recommendations cannot be drawn from retrospective studies and only the ongoing, well designed prospective trials will provide definitive answers to this important question.

Conclusions

In this study, a statistically significant and clinically meaningful benefit for the addition of adjuvant CT to endocrine treatment in 70-gene high risk patients in the adjuvant setting has been shown. There appears to be no evidence for a similar benefit for the 70-gene low risk patients and these patients are at such a low risk of recurrence and cancer-related death, that addition of CT may not be justified. ET alone seems to be the optimal treatment for this group of patients. It seems reasonable to use multigene assays whenever indicated in hormone receptor-positive patients for improved decision-making regarding the role of adding adjuvant CT to hormonal treatment.

Acknowledgments

This work was supported by the Austrian Society of Surgery and Agendia BV. Both provided unrestricted educational grants for the work of M. Knauer. We are indebted to Femke de Snoo, MD PhD for critically reading the manuscript and providing helpful comments and to Marleen Kok for providing part of the data used for our analyses.

References

1. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; 365: 1687-1717.
2. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. Breast Cancer-v.1.2010. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf.
3. Goldhirsch A, Ingle JN, Gelber RD, et al. Thresholds for therapies: highlights of the St. Gallen International Expert Consensus on the primary therapy of early breast cancer 2009. *Ann Oncol* 2009; 20:1319-1329.
4. Dowsett M, Goldhirsch A, Hayes DF, et al. International Web-based consultation on priorities for translational breast cancer research. *Breast Cancer Res* 2007; 9: R81.
5. Van't Veer LJ, Dai H, Van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; 415: 530-536.
6. Van de Vijver MJ, He YD, Van 't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; 347: 1999-2009.
7. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; 351: 2817-2826.
8. Habel LA, Shak S, Jacobs MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res* 2006; 8: R25.
9. Sotiriou C, Wirapati P, Loi S, et al. Gene expression profiling in breast cancer: understanding the molecular basis of histologic grade to improve prognosis. *J Natl Cancer Inst* 2006; 98: 262-272.
10. Ma XJ, Wang Z, Ryan PD, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell* 2004; 5: 607-616.
11. Ma XJ, Salunga R, Dahiya S, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. *Clin Cancer Res* 2008; 14: 2601-2608.
12. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006; 24: 3726-3734.
13. Buyse M, Loi S, Van 't Veer L, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst* 2006; 98: 1183-1192.
14. Bueno de Mesquita JM, Linn SC, Keijzer R et al. Validation of 70-gene prognosis signature in node-negative breast cancer. *Breast Cancer Res Treat* 2009; 117: 483-495.
15. Knauer M, Wenzl E, Rutgers EJT, Linn SC, Van 't Veer LJ. Gene expression profiling in breast cancer - design of a pooled database to address open questions. *Eur Surg* 2009; 41: 221-227.
16. Bueno de Mesquita JM, Van Harten WH, Retel VP, et al. Use of 70-gene signature to predict prognosis of patients with node-negative breast cancer: a prospective community-based feasibility study (RASTER). *Lancet Oncol* 2007; 8: 1079-1087.
17. Mook S, Schmidt MK, Viale G, et al. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. *Breast Cancer Res Treat* 2009; 116: 295-302.

18. Mook S, Schmidt MK, Weigelt B, et al. The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. *Ann Oncol* 2010; 21: 717-722.
19. Glas AM, Floore A, Delahaye LJ, et al. Converting a breast cancer microarray signature into a high-throughput diagnostic test. *BMC Genomics* 2006; 7: 278-287.
20. Ioannidis JP. Gene expression profiling for individualized breast cancer chemotherapy: success or not? *Nat Clin Pract Oncol* 2006; 3: 538-539.
21. Simmonds MC, Higgins JP, Stewart LA, et al. Meta-analysis of individual patient data from randomized trials: a review of methods used in practice. *Clin Trials* 2005; 2: 209-217.
22. Cardoso F, Van't Veer L, Rutgers E, et al. Clinical application of the 70-gene profile: the MINDACT trial. *J Clin Oncol* 2008; 26: 729-735.
23. Sparano JA. TAILORx: trial assigning individualized options for treatment (Rx). *Clin Breast Cancer* 2006; 7: 347-350.
24. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol* 2010; 11: 55-65.
25. Sotiriou C and Pusztai L. Gene-expression signatures in breast cancer. *N Engl J Med* 2009; 360: 790-800.
26. Ayers M, Symmans WF, Stec J, et al. Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. *J Clin Oncol* 2004; 22: 2284-2293.
27. Bonnefoi H, Potti A, Delorenzi M, et al. Validation of gene signatures that predict the response of breast cancer to neoadjuvant chemotherapy: a substudy of the EORTC 10994/BIG 00-01 clinical trial. *Lancet Oncol* 2007; 8: 1071-1078.
28. Hess KR, Anderson K, Symmans WF, et al. Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. *J Clin Oncol* 2006; 24: 4236-4244.
29. Liedtke C, Hatzis C, Symmans WF, et al. Genomic grade index is associated with response to chemotherapy in patients with breast cancer. *J Clin Oncol* 2009; 27: 3185-3191.
30. Chang JC, Wooten EC, Tsimelzon A, et al. Gene expression profiling for the prediction of therapeutic response to docetaxel in patients with breast cancer. *Lancet* 2003; 362: 362-369.
31. Dressman HK, Hans C, Bild A, et al. Gene expression profiles of multiple breast cancer phenotypes and response to neoadjuvant chemotherapy. *Clin Cancer Res* 2006; 12: 819-826.
32. Folgueira MA, Carraro DM, Brentani H, et al. Gene expression profile associated with response to doxorubicin-based therapy in breast cancer. *Clin Cancer Res* 2005; 11: 7434-7443.
33. Gianni L, Zambetti M, Clark K, et al. Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. *J Clin Oncol* 2005; 23: 7265-7277.
34. Iwao-Koizumi K, Matoba R, Ueno N, et al. Prediction of docetaxel response in human breast cancer by gene expression profiling. *J Clin Oncol* 2005; 23: 422-431.
35. Park S, Shimizu C, Shimoyama T, et al. Gene expression profiling of ATP-binding cassette (ABC) transporters as a predictor of the pathologic response to neoadjuvant chemotherapy in breast cancer patients. *Breast Cancer Res Treat* 2006; 99: 9-17.

36. Potti A, Dressman HK, Bild A, et al. Genomic signatures to guide the use of chemotherapeutics. *Nat Med* 2006; 12: 1294-1300.
37. Thuerigen O, Schneeweiss A, Toedt G, et al. Gene expression signature predicting pathologic complete response with gemcitabine, epirubicin, and docetaxel in primary breast cancer. *J Clin Oncol* 2006; 24: 1839-1845.
38. Straver ME, Glas AM, Hannemann J, et al. The 70-gene signature as a response predictor for neoadjuvant chemotherapy in breast cancer. *Breast Cancer Res Treat* 2010; 119: 551-558.