Gene Expression Profiling in Breast Cancer and Its Effect on Therapy Selection in Early-Stage Breast Cancer

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ABSTRACT

Breast cancer is a heterogeneous disease. The purpose of adjuvant therapy for early-stage breast cancer is to provide maximum benefit with minimum side effects and not to under-treat or over-treat. The clinical progresses of patients with the same clinical and pathological characteristics who are given similar treatments may show major differences. This fact indicates that the prognostic and predictive factors that we have used until recent years for therapy selection are not really sufficient, we need new markers, every disease and every individual are unique and that treatment should be individualized. The gene expression profiling, which has come into clinical use in recent years, is beneficial in therapy selection for luminal breast cancer cases. A differentiation can be made among patients for whom only endocrine treatment would be adequate and those who should also receive chemotherapy in addition to endocrine treatment. Several new gene expression analysis studies targeted at gaining the ability to determine drug selection in chemotherapy, endocrine treatment and neo-adjuvant therapy are also currently ongoing. The staging system for new breast cancer that is to be published in the year 2018 also includes gene expression analyses within the prognostic panel and the stage changes depending on the result. The statement 'Treat the patient, not the disease.' is becoming increasingly entrenched in our clinical practice. This article briefly summarizes the gene expression profiles, which are validated and used in the selection of therapy for early-stage breast cancer.

Keywords: Early breast cancer, molecular subtypes, gene-expression profiling, prognosis, staging

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Introduction

Breast cancer is the type of cancer with the highest incidence rate among women globally and it ranks the first among reasons for death due to cancer in women (1). Appropriate selection and administration of therapies according to the patient are important for not only prolonging disease-free survival and overall survival, but also for preventing late complications (such as anthracycline-related cardiac problems, myelodysplastic syndrome, leukemia and taxane-associated neuropathy) (2).

It is a standard approach to administer adjuvant chemotherapy (CT) and/or hormonal treatment (HT) to reduce the risk for metastasis according to the histological, pathological and immunohistochemical staining characteristics of the tumor following surgical treatment of early-stage breast cancer (3-5). The strongest predictors in determining the risk for metastasis are as follows: tumor diameter, histological grade, axillary lymph nodes (ALN) metastasis and hormone receptors (HR) and HER2 status as well as Ki-67 proliferation index (6). In recent years, molecular sub-types (intrinsic sub-types) of breast cancer have been described and therapy selection is done according to these sub-types (7-10). Breast cancers are divided into four sub-groups as per their molecular characteristics (11, 12): Luminal, HER2-positive, basal and normal-like (Table 1). CT is necessary for adjuvant treatment of basal-type and HER2-positive breast cancer cases.

Luminal tumors are HR-positive breast cancers that constitute approximately 60-80% of all breast cancer cases (8, 11, 12). Luminal tumors are divided into two groups as A and B: Their characteristics are summarized in Table 2. While luminal A tumors are very sensitive to HT, it may be necessary to use CT and HT together in luminal B tumors. The above-specified classical predictors are not

Table 1. Breast cancer molecular subtypes (Modified from 11)

Intrinsic subtype	IHC* status	Grade	Prognosis	Prevalence
Luminal A	ER+/PR+;HER2-;Ki67 low	1/2	Good	40%
Luminal B	ER+/PR+;HER2-;Ki67 high ER+/PR+;HER2+;Ki67 any	2/3 2/3	Intermediate Intermediate	20%
HER2 overexpression	ER-/PR-;HER2+;Ki67 any	2/3	Poor	12 to 21%
Basal	ER-/PR-;HER2-;basal marker+	3	Poor	11 to 23%
Normal-like	ER+/PR+;HER2-Ki67 any	1/2/3	Intermediate	3 to 10%

*IHC: Immuno-Histo-Chemical Staining; ER: Estrogen Receptor; PR: Progesteron Receptor

Table 2: Characteristics of Luminal Subtypes Breast Cancers (Modified from 8)

	Luminal A	Luminal B
Incidence %	40	20
ER-related genes	High	Relatively low
HER2-related genes	Low	Variable
TP53 mutation	12%	29%
Proliferation-related genes	Low	High
Prognostic significance	Good	Poor
Prediction to endocrine therapy	Highly sensitive	Relatively less sensitive
Prediction to cytotoxic therapy	Less sensitive	Less sensitive

ER: estrogen receptor

sufficient to distinguish between the luminal A and B sub-groups in HR-positive, HER2-negative breast cancer and does not account for different clinical progresses of luminal type breast cancer patients who are in the same stage and receive similar treatments. In order to explain this difference and better differentiate between patients who may not require chemotherapy as part of adjuvant therapy and those who need to have addition of CT to their hormone therapy, various gene expression analyses have been and are being studied retrospectively and prospectively.

Gene Expression Profiling

Amsterdam 70-gene Profile- MammaPrint dx Test (Table 3)

The first one of these tests is MammaPrint dx 70-gene expression analysis, which was developed by the Netherlands Cancer Institute. They identified 70 genes which differentiate between patients diagnosed with breast cancer as good profile and poor profile depending on the risk of developing metastasis within 5 years as of diagnosis. This gene profile was developed based on a gene study conducted with 78 ALN-negative patients below the age of 55 and diagnosed with invasive breast cancer a tumor size <5 cm (13, 14). The test is conducted using the micro-array-based gene expression profiling technique. Fresh tissue sample or frozen archival material and formalin-fixed paraffin-embedded (FFPE) material are used for the test (9, 15). Several studies have demonstrated that MammaPrint is an independent prognostic factor in patients with ALN-negative breast cancer (16-18). It has been

seen that 35% of patients that seemed to have high risk disease actually had low risk and 14% of patients that seemed to be in the low risk group actually had high risk in this program as compared to the adjuvant online program (19).

The MammaPrint test is recommended for early breast cancer of all ages with tumor size <5 cm, with the ALN 0-3 positive cases, and estrogen receptor (ER)-positive or negative tumors (8). It was approved by Food and Drug Administration (FDA) in 2007 for marketing as a prognostic test, but not to select therapy or predict response to therapy (15). Results are reported as low risk (13% chance of developing distant metastases at 10 years without adjuvant treatment) or high risk (56% chance of developing distant metastases at 10 years without adjuvant treatment).

For the prospective validation of the test, an international, randomized, Phase-3 MINDACT study (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy-EORTC 10041/BIG 3-04 study) has been scheduled (20). In this study, 6693 patients with early-stage breast cancer (HR+, Nod 1-3 positive or negative, HER2-) were recruited. The genomic risks (using the 70-gene signature) and clinical-pathological risks (using a modified version of Adjuvant Online) of the patients were identified and the aim has been set as comparing their effectiveness in the adjuvant therapy selection. CT was not provided for the group with low genomic and clinical risk. CT was provided for the group with high genomic and clinical-pathological risk. In patients with discordant risk results, either the genomic risk or the clinical-pathological risk was used to determine the use of chemotherapy and hormone therapy or only hormone therapy. The primary aim of the study was to determine whether patients with high clinical risk and low genomic risk had requirement for CT. According to the assessment of 1550 patients with high clinical risk and low genomic risk, metastasis-free survival rate was found to be 94.7% in patients not receiving CT, which was 1.5% lower than the patients that were on CT. The rates of distant metastasis-free survival were found to be similar among patients that were node-negative or positive, ER-positive and HER2-. Based on these results, the researchers concluded that 46% of the patients with low genomic risk and high clinical risk did not require CT.

MammaPrint is featured as a prognostic parameter in the St Gallen and ESMO (European Society of Medical Oncology) guidelines (3, 21).

In the 8th Breast Cancer Staging System of American Joint Committee on Cancer (AJCC), which is to be published in 2018, gene expression profiling is included in breast cancer staging as a prognostic panel (22).

Table 3: Gene Expression Tests for Predicting Clinical Outcomes in Patients with HR positive, HER2 negative, Node 0-3 Positive Early Stage Breast Cancer (7, 9, 22, 34, 40)

	Oncotype DX	MammaPrint	PAM50-risk of recurrence score	Breast Cancer Index	EndoPredict
Manufacturer	Genomic Health (Redwood City, CA)	Agendia (Irvine, CA)	NanoString Technologies (Seattle, WA)	bioTheranostics	Myriad/ Sividon Diagnostics
Tissue Sample	FFPE	Fresh, frozen, or FFPE	FFPE	FFPE	FFPE
No of genes	16 cancer 5 control	70	50 cancer 22 control/ housekeeping + tumor size	MGI-5 cell cycle genes; H/I-Gene expression ratio	8 cancer 3 control
Technology	Quantitative RT-PCR	Microarrays	Quantitative RT-PCR	Quantitative RT-PCR	Quantitative-RT-PCR
Predictive	+	-	-	+	-
Prognostic	+	+	+	+	+
Eligible patients	ER+ and HER2-, T1/2 0-3 nodes f	Stage I-and II breast cancer	ER+, stage I/II 0-3 nodes	ER+ HER2- Node-	ER+ HER2-
Measure/ Categories	RS Low <18 Intermediate 18-31 High >31	Good risk and poor risk intrinsic subtype	ROR:Ten year probability of distant recurrence Low <10% Intermediate 10-20% High >20% Intrinsic subtype	Low, intermediate and high risk	The test result is composed of the 'molecular fingerprint' of a tumor in combination with the established prognostic parameters nodal status and tumor size
Strength of ASCO recommendation	Strong for N0 Moderate for N+	Moderate for N0 and N+	Strong for N0 Moderate for N+	Moderate for N0 Strong for N+ (don't use for N+)	Moderate for N0 and N+
8th AJCC breast cancer staging manual (when available as stage modifiers).	For patients with HR- positive, HER2 negative, and ALN negative tumors, Oncotype Dx recurrence score less than 11, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0, and the tumor is staged using the AJCC prognostic stage group table as stage I (Level of Evidence I).	For patients with HR-positive, HER2 negative, and ALN negative tumors, a MammaPrint low-risk score, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).	For patients with HR-positive, HER2 negative, and ALN negative tumors, a PAM50 ROR score in the low- range, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).	For patients with HR- positive, HER2 negative, and ALN negative tumors, a BCI in the low-range, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).	For patients with HR-positive, HER2 negative, and ALN negative tumors, Endopredict low-risk score, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).

AJCC: American Joint Committee on Cancer; ALN: axillary lymph node; ASCO: American Society of Clinical Oncology; FFPE: formaline-fixed paraffin embedded; RT-PCR: reverse transcriptase-polymerase chain reaction; ER: estrogen receptor; HR: hormone receptor; BCI: Breast Cancer Index; ROR: Risk of Recurrence

One of these panels is MammaPrint (when available as stage modifiers): For patients with HR-positive, HER2 negative, and ALN negative tumors, a MammaPrint low-risk score, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).

21-Gene Recurrence Score assay (Oncotype DX) (Table 3)

The Oncotype DX Breast Cancer Assay is a commercially available reverse transcriptase-polymerase chain reaction (RT-PCR) based signature. It evaluates the mRNA levels of 21 genes (16 cancer-related genes and 5 reference genes) (8, 9, 15). The expression of these 21 genes is reported as a single Recurrence Score (RS), which ranges between 0 and 100. The test is routinely performed on FFPE tissue specimens.

Patients are divided into 3 risk groups depending on the risk for distant metastasis in ten years: 1- Low risk (RS<18) 2- Intermediate risk (RS 18-30) 3- High risk (RS>30)

The clinical validation of Oncotype DX was originally completed in 2 retrospective studies (National Surgical Adjuvant Breast and Bowel Project-NSABP B-14 and B-20 studies) (23, 24): In the NSABP B-14 study, patients (HR-positive disease with negative axillary nodes) were randomized to the tamoxifen versus placebo arms for 5 years (23). The distant metastasis-free survival rates of the patients as per their risk groups were as follows: Low risk group (RS<18; 10-year risk for distant metastasis: 6.8%); intermediate risk group (RS:18-30; 10-year risk for distant metastasis: 14.3%) and high risk group (RS>30; 10-year risk for distant metastasis: 30.5). In conclusion, Oncotype DX was inter-

puted as being a 'predictor of distant relapse in ER+ node negative disease.' These results were also tested in the NSABP B-20 study (HR positive disease with negative axillary nodes; adjuvant tamoxifen versus CMF + tamoxifen) and the contribution of CT as per the risk groups was investigated (24): It was seen that the addition of CT to tamoxifen for patients in the low risk group decreased the 10-year risk for distant metastasis by only 1.1% while the addition of CT to tamoxifen for patients in the high risk group reduced the 10-year risk for distant metastasis significantly by 27.6%. The benefit of the addition of CT to hormonal therapy in the intermediate risk group was not showed to be clinically significant.

The action to be taken for patients in the intermediate risk group could not be completely elucidated. Should it be only HT or CT and HT? In order to shed light onto this question, a prospective randomized study was initiated in the year 2006 (Trial Assigning Individualized Options for Treatment-TAILORx; prospective clinical validation study) (25). In this study, 10,253 patients (HR-positive and HER2-negative with negative axillary nodes) from 6 countries and 900 study sites were included between the years 2006 and 2010. The patients were divided into groups as follows: RS<11 low risk group (only endocrine treatment), RS 11-25 intermediate risk group (divided into two arms: only HT and CT + HT) and RS>25 high risk group (CT and HT). As part of HT, tamoxifen or aromatase inhibitor (AI) or tamoxifen followed by AI were administered for 5 years and tamoxifen or AI along with ovarian suppression were used in 3% of the patients. During the ESMO 2015 meeting, the results of the low-risk group (15.9% of all patients; 1626 patients) were presented as follows: 5-year invasive disease free survival: 93.8%; recurrence-free survival: 98.7%; distant recurrence-free survival: 99.3% and overall survival: 98%. Recurrence events were uncommon regardless of the histologic grade, tumor size and were not significantly affected by younger age at diagnosis in this low risk group.

The results of the study also prospectively showed that only adjuvant HT was sufficient for patients with low risk according to the 21 gene expression analysis (Level IA evidence). It is expected that the results of the intermediate risk group will be announced within the year 2017.

The prognostic and predictive validity of Oncotype DX was also retrospectively evaluated in 4 randomized phase-3 studies [SWOG 8814, ATAC (Adjuvant Tamoxifen or Anastrozole), NSABP-B28 and ECOG 2197 studies] including ALN positive and HR-positive cases (26-30). It was seen that CT was beneficial in distinguishing node-positive patients who would benefit from CT (Predictor of likelihood of chemotherapy benefit in ER+ Node positive disease). Its prospective validation in the node-positive patients was demonstrated in the West German Study Group Plan B Randomized Phase-3 study, the Clalit Registry study conducted in Israel and the SEER real-life observational study (31-33). In all these studies, the 5-year survival rate of patients in the low risk group is >95%.

Other ongoing trials (RxPONDER and OPTIMA) are evaluating whether adjuvant CT is beneficial in patients with HR-positive, HER2-negative breast cancer with 1 to 3 positive ALNs and a RS of 25 or less (7, 8).

Oncotype DX is included as a prognostic and predictive tests in the ESMO, St Gallen, NCCN (National Comprehensive Cancer Network; includes 1 to 3 positive nodes) and ASCO (American Society of Clinical Oncology; node negative only) guidelines (3, 4, 21, 34).

The Oncotype DX staging system has been included in the 8th breast staging system by AJCC (when available as stage modifiers) (22): For patients with HR-positive, HER2-negative and ALN-negative tumors, Oncotype DX recurrence score less than 11, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0, and the tumor is staged using the AJCC prognostic stage group table as stage I (Level of Evidence I).

Predictor analysis of microarray 50 risk of recurrence score (PAM50-ROR) (Table 3)

The PAM50 is a test that uses 50 classifier genes and 5 control genes. The microarray technique is employed and study is done on FFPE tissues with quantitative RT-PCR technology (8, 9, 15). Along with the tumor diameter and four main intrinsic sub-types are provided along with the risk of recurrence (ROR). The PAM50 score is reported on a 0-100 scale (ROR score of risk of recurrence), which is correlated with the probability of distant recurrence at ten years for women with HR positive, early-stage node-negative or node 1-3 positive breast cancer. Patients are divided into high (>20%), intermediate (10 to 20%) and low (<10%) risk groups. It was retrospectively tested in the ATAC and ABCSG-8 (Austrian Breast Cancer Study Group 8) studies and demonstrated to be an important prognostic indicator for both ALN-negative and ALN-positive patients in all sub-groups (35, 36). It is an FDA-approved test (9, 34).

It has been included in the 8th breast cancer staging system by the AJCC (when available as stage modifiers) (22): For patients with HR-positive, HER2-negative and ALN-negative tumors, a PAM50 ROR score in the low-range, regardless of T size, places the tumor in the same prognostic category as T1a-T1b N0M0 (Level of Evidence II).

Breast Cancer Index (BCI) (Table 3)

Breast Cancer Index is a combination of molecular grade index (MGI) and HOXB13-to-IL17BR expression ratio (H:I ratio). Studies conducted have shown that it is effective in anticipating treatment response and prognosis in ER-positive tumors (37, 38). Three risk groups are identified: low, intermediate and high risk. Its clinical usability is still being investigated. ASCO states that it can be used in making a decision for adjuvant therapy in HR-positive, HER2-negative and node-negative disease while it is not recommended to be used in node-positive disease (34).

EndoPredict (Table 3)

It involves RNA-based analysis of 11 genes (8 cancer related and 3 reference genes). Its prognostic value was validated using the data from ABCSG-6 and ABCSG-8 trials (39). ASCO states that it can be used in making a decision for adjuvant therapy in HR-positive, HER2-negative and node-negative disease while it is not recommended to be used in node-positive disease (34). It is a test which can also be used to make a decision for prolonged adjuvant therapy.

Breast Cancer Index and EndoPredict (when available as stage modifiers) tests are also included as part of the prognostic panel in the 8th staging booklet (Level of evidence II) (22) (Table 3).

Other assays include the Rotterdam 76-gene signature, genomic grade index, molecular grade index, etc. There are not sufficient data about the prognostic significance of these arrays (9, 15, 34, 40, 41). Further studies are needed.

Conclusion

Gene expression analyses are beneficial in determining the prognosis and selecting therapy for luminal type breast cancers (HER2-negative, HR-positive). Even though these tests are costly, studies performed have shown that they are actually cost-efficient (42, 43). They are included in reimbursement schemes in the USA and Europe whereas they are not included in the reimbursement program in Turkey and many other countries. Therefore, studies can be conducted only on a limited group of patients. Studies conducted in Turkey have demonstrated that Oncotype DX has significantly correlated with PR and Ki-67 score of the tumor, and has a significant contribution to determining the therapy selection (44, 45). In another study from Turkey, Oncotype DX test was found as cost-effective in patients with early stage breast cancer (46).

It should also be kept in mind that gene expression analyses may yield false results in rarely seen tumors such as breast cancers showing neuroendocrine differentiation and in mixed morphologies (47). Furthermore, the stromal cells and inflammatory cells around the tumor tissue and the normal breast tissue are not included in the analysis. The ratio of non-neoplastic cells in the analysis may change the expression profile and the prognostic signature. In the second-generation gene expression analyses, myoepithelial and stromal cells are also assessed in addition to the epithelial cancer cells (8).

There are no gene expression tests available yet to determine the therapy selection for other intrinsic types of breast cancers. Various gene expression analyses and second generation gene expression analysis studies are ongoing with the aims of determining the drug selection in endocrine treatment, selecting the agent to be used in chemotherapy and predicting treatment to neoadjuvant therapy (8). Technological advancements and developments in the field of molecular biology and genetics will enable us to provide individualized therapies for our patients.

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References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Pattern in GLOBOCAN 2012. *Int J Cancer* 2015; 136: 359-386. (PMID: 25220842) [\[CrossRef\]](#)
2. Davitson NE, Stearns V. Adjuvant Chemo Endocrine Therapy In. Harris JR, Lippman ME, Morow M, Osborne CK(eds). *Disease of the Breast*, 5th ed. Wolter Kluwer Health, Philadelphia, 2014.pp.649-668.
3. Coates AS, Winer EP, Goldhisch A, Gelber RD, Gnant M, Piccart-Gebhart M, Thürlimann B, Senn HJ, Panel Members. Tailoring Therapies-Improving the Mangement of early Breast Cancer: St Gallen International Expert Consensus on the Primary Therapy of early Breast Cancer 2015. *Ann Oncol* 2015; 26: 1533-1546. (PMID: 25939896) [\[CrossRef\]](#)
4. National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline in Oncology. Breast Cancer Version 2. 2017- April 6, 2017. Available from: NCCN.org
5. Gnant M, Harbeck N, Thomssen C. St Gallen/Vienna 2017: A Brief Summary of the Consensus Discussion about Escalation and De-Escalation of Primary Breast Cancer Treatment. *Breast Care* 2017; 12: 102-107. (PMID: 28559767) [\[CrossRef\]](#)
6. Masood S. Prognostic/Predictive Factors in Breast Cancer. *Clin Lab Med* 2005; 25: 809-825. (PMID: 16308094) [\[CrossRef\]](#)
7. Henry NL, Bedard PL, DeMichele A. Standart and Genomic Tools for Decision Support in Breast Cancer Treatment. *Am Soc Clin Oncol Educ Book* 2017; 37: 106-115. (PMID: 28561710). [\[CrossRef\]](#)
8. Verma A, Kaur J, Mehta K. Molecular Oncology Update: Breast Cancer Gene Expression Profiling. *Asian J Oncol* 2015; 1: 65-72. [\[CrossRef\]](#)
9. Rosa M. Advances in the Molecular Analysis of Breast Cancer: Pathway Toward Personalized Medicine. *Cancer Control* 2015; 22: 211-219. (PMID: 26068768) [\[CrossRef\]](#)
10. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. *Nature* 2000; 406: 747-752. (PMID: 10963602) [\[CrossRef\]](#)
11. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, Shi B. Breast Cancer Intrinsic Subtype Classification, Clinical Use and Future Trends. *Am J Cancer Res* 2015; 5: 2929-2943. (PMID: 26693050)
12. Sørli T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Borresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001; 98: 10869-10874. (PMID: 11553815) [\[CrossRef\]](#)
13. van de Vijver MJ, He YD, van 't Veer LJ, Dai H, Hart AA, Voskuil DW, Schreiber GJ, Peterse JL, Roberts C, Marton MJ, Parrish M, Atsma D, Witteveen A, Glas A, Delahaye L, van der Velde T, Bartelink H, Rodenhuis S, Rutgers ET, Friend SH, Bernards R. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002; 347: 1999-2009. (PMID: 12490681) [\[CrossRef\]](#)
14. van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002; 415: 530-536. (PMID: 11823860) [\[CrossRef\]](#)
15. Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: Classification, prognostication, and prediction. *Lancet* 2011; 378: 1812-1823. (PMID: 22098854) [\[CrossRef\]](#)
16. Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, d'Assignies MS, Bergh J, Lidereau R, Ellis P, Harris A, Bogaerts J, Therasse P, Floore A, Amakrane M, Piette F, Rutgers E, Sotiriou C, Cardoso F, Piccart MJ; TRANSBIG Consortium. 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. (PMID: 16954471) [\[CrossRef\]](#)
17. Mook S, Schmidt MK, Weigelt B, Kreike B, Eekhout I, van de Vijver MJ, Glas AM, Floore A, Rutgers EJ, van 't Veer LJ. 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. (PMID: 19825882) [\[CrossRef\]](#)
18. Knauer M, Mook S, Rutgers EJ, Bender RA, Hauptmann M, van de Vijver MJ, Koornstra RH, Bueno-de-Mesquita JM, Linn SC, van 't Veer LJ. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. *Breast Cancer Res Treat* 2010; 120: 655-661 (PMID: 20204499) [\[CrossRef\]](#)
19. Turaga K, Acs G, Laronga C. Gene Expression Profiling in Breast Cancer. *Cancer Control* 2010; 17: 177-182. (PMID: 20664515) [\[CrossRef\]](#)
20. Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, Pierga JY, Brain E, Causeret S, DeLorenzi M, Glas AM, Goulinopoulos V, Goulioti T, Knox S, Matos E, Meulemans B, Neijenhuis PA, Nitz U, Passalacqua R, Ravdin P, Rubio IT, Saghatian M, Smilde TJ, Sotiriou C, Stork L, Strahle C, Thomas G, Thompson AM, van der Hoeven JM, Vuylsteke P, Bernards R, Tryfonidis K, Rutgers E, Piccart M; MINDACT Investigators. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med* 2016; 375: 717-729. (PMID: 27557300) [\[CrossRef\]](#)

21. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, Zackrisson S, Cardoso F; ESMO Guidelines Committee. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015; 26: 8-30. (PMID: 28428927). [\[CrossRef\]](#)
22. Giuliano AE, Connolly JL, Edge SB, Mittendorf EA, Rugo HS, Solin LJ, Weaver DL, Winchester DJ, Hortobagyi GN. Breast Cancer-Major Changes in the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual. *CA Cancer J Clin* 2017; 67: 290-303. (PMID: 28294295)
23. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M, Baehner FL, Walker MG, Watson D, Park T, Hiller W, Fisher ER, Wickerham DL, Bryant J, Wolmark N. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004; 351: 2817-2826. (PMID: 15591335) [\[CrossRef\]](#)
24. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, Cronin M, Baehner FL, Watson D, Bryant J, Costantino JP, Geyer CE, Jr, Wickerham DL, Wolmark N. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006; 24: 3726-3734. (PMID: 16720680) [\[CrossRef\]](#)
25. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, Geyer CE Jr, Dees EC, Perez EA, Olson JA Jr, Zujewski J, Lively T, Badve SS, Saphner TJ, Wagner LJ, Whelan TJ, Ellis MJ, Paik S, Wood WC, Ravdin P, Keane MM, Gomez Moreno HL, Reddy PS, Goggins TF, Mayer IA, Brufsky AM, Toppmeyer DL, Kaklamani VG, Atkins JN, Berenberg JL, Sledge GW. Prospective validation of a 21-gene expression assay in breast cancer. *N Engl J Med* 2015; 373: 2005-2014. (PMID: 26412349) [\[CrossRef\]](#)
26. Albain KS, Barlow WE, Shak S, Hortobagyi GN, Livingston RB, Yeh IT, Ravdin P, Bugarini R, Baehner FL, Davidson NE, Sledge GW, Winer EP, Hudis C, Ingle JN, Perez EA, Pritchard KI, Shepherd L, Gralow JR, Yoshizawa C, Allred DC, Osborne CK, Hayes DF. 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. (PMID: 20005174) [\[CrossRef\]](#)
27. Dowsett M, Cuzick J, Wale C, Forbes J, Mallon EA, Salter J, Quinn E, Dunbier A, Baum M, Buzzard A, Howell A, Bugarini R, Baehner FL, Shak S. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol* 2010; 28: 1829-1834. (PMID: 20212256) [\[CrossRef\]](#)
28. Mamounas EP, Tang G, Paik S, Baehner FL, Liu Q, Jeong J-H, Kim S-R, Butler SM, Jamshidian F, Cherbavaz DB, Shak S, Julian TB, Lembersky BC, Wickerham DL, Costantino JP, Wolmark N. Prognostic impact of the 21-gene recurrence score (RS) on disease-free and overall survival of node-positive, ER-positive breast cancer patients (pts) treated with adjuvant chemotherapy: Results from NSABP B-28. *J Clin Oncol* 2012; 30: 27 suppl 1-1.
29. Solin LJ, Gray R, Goldstein LJ, Recht A, Baehner FL, Shak S, Badve S, Perez EA, Shulman LN, Martino S, Davidson NE, Sledge GW Jr, Sparano JA. Prognostic value of biologic subtype and the 21-gene recurrence score relative to local recurrence after breast conservation treatment with radiation for early stage breast carcinoma: results from the Eastern Cooperative Oncology Group E2197 study. *Breast Cancer Res Treat* 2012; 134: 683-692. (PMID: 22547108) [\[CrossRef\]](#)
30. Brufsky AM. Predictive and Prognostic Value of the 21-Gene Recurrence Score in Hormone Receptor-positive, Node Positive Breast Cancer. *Am J Clin Oncol* 2014; 37: 404-410. (PMID: 24853663) [\[CrossRef\]](#)
31. Stemmer SM, Klang SH, Ben-Baruch N, Geffen DB, Steiner M, Soussan-Gutman L, Merling S, Svedman C, Rizel S, Lieberman N. The impact of the 21-gene Recurrence Score assay on clinical decision-making in node-positive (up to 3 positive nodes) estrogen receptor-positive breast cancer patients. *Breast Cancer Res Treat* 2013; 140: 83-92. (PMID: 23801158) [\[CrossRef\]](#)
32. Gluz O, Nitz UA, Christgen M, Kates RE, Shak S, Clemens M, Kraemer S, Aktas B, Kuemmel S, Reimer T, Kusche M, Heyl V, Lorenz-Salehi F, Just M, Hofmann D, Degenhardt T, Liedtke C, Svedman C, Wuerstlein R, Kreipe HH, Harbeck N. West German Study Group Phase III Plan B Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment. *J Clin Oncol* 2016; 34: 2341-2349. (PMID: 26926676) [\[CrossRef\]](#)
33. Roberts MC, Miller DP, Shak S, Petkov VI. Breast cancer-specific survival in patients with lymph node-positive hormone receptor-positive invasive breast cancer and Oncotype DX Recurrence Score results in the SEER database. *Breast Cancer Res Treat* 2017; 163: 303-310. (PMID: 28243896) [\[CrossRef\]](#)
34. Harris LN, Ismaila N, McShane LM, Andre F, Collyar DE, Gonzales-Angula AM, Hammond EH, Kuderer NM, Liu MC, Mennel RG, Poznak CV, Bast RC, Hayes DF. Use of Biomarkers to Guide Decision on Adjuvant Systemic Therapy for Women With Early Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2016; 34: 1134-1150. (PMID: 26858339) [\[CrossRef\]](#)
35. Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, Mlineritsch B, Kwasny W, Knauer M, Singer C, Jakesz R, Dubsky P, Fitzal F, Bartsch R, Steger G, Balic M, Ressler S, Cowens JW, Strohoff J, Ferree S, Schaper C, Liu S, Fesl C, Nielsen TO; Austrian Breast and Colorectal Cancer Study Group. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol* 2014; 25: 339-345. (PMID: 24347518) [\[CrossRef\]](#)
36. Gnant M, Sestak I, Filipits M, Dowsett M, Balic M, Lopez-Knowles E, Greil R, Dubsky P, Stoeger H, Rudas M, Jakesz R, Ferree S, Cowens JW, Nielsen T, Schaper C, Fesl C, Cuzick J. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of recurrence score and intrinsic subtype. *Ann Oncol* 2015; 26: 1685-1691. (PMID: 25935792) [\[CrossRef\]](#)
37. Ma XJ, Salunga R, Dahiya S, Wang W, Carney E, Durbecq V, Harris A, Goss P, Sotiriou C, Erlander M, Sgroi D. 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. (PMID: 18451222) [\[CrossRef\]](#)
38. Jansen MP, Sieuwerts AM, Look MP, Ritscher K, Meijer-van Gelder ME, van Staveren IL, Klijn JG, Foekens JA, Berns EM. HOXB13-to-IL17BR expression ratio is related with tumor aggressiveness and response to tamoxifen of recurrent breast cancer: A retrospective study. *J Clin Oncol* 2007; 25: 662-668. (PMID: 17308270) [\[CrossRef\]](#)
39. Filipits M, Rudas M, Jakesz R, Dubsky P, Fitzal F, Singer CE, Dietze O, Greil R, Jelen A, Sevela P, Freibauer C, Müller V, Jänicke F, Schmidt M, Kölbl H, Rody A, Kaufmann M, Schroth W, Brauch H, Schwab M, Fritz P, Weber KE, Feder IS, Hennig G, Kronenwett R, Gehrmann M, Gnant M, EP Investigators. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res* 2011; 17: 6012-6020. (PMID: 21807638) [\[CrossRef\]](#)
40. Syed MP, Kolluri S, Goffin JV, Tripathy D. Clinical Decision Making in Stage I and II Breast Cancer Patients Based on Gene Profiling. *Am J Hematol/Oncol* 2016; 12: 7-16.
41. Scope A, Essat M, Pandor A, Rafia R, Ward SE, Wyld L, Cross S, Woods HB. Gene expression profiling and expanded immunohistochemistry Tests to Guide Selection of Chemotherapy Regimens in Breast Cancer Management: A Systematic Review. *Int J Technol Assess Health Care* 2017; 10: 1-14. (PMID: 28486999) [\[CrossRef\]](#)
42. Kondo M, Hoshi SL, Yamanaka T, Ishiguro H, Toi M. Economic evaluation of the 21-gene signature (Oncotype DX) in lymph node-negative/positive, hormone receptor-positive early-stage breast cancer based on Japanese validation study (JBCRG-TR03) Breast Cancer Res Treat 2011; 127: 739-749. (PMID:21082239) [\[CrossRef\]](#)
43. Retèl VP, Joore MA, Knauer M, Linn SC, Hauptmann M, Harten WH. Cost-effectiveness of the 70-gene signature versus St. Gallen guidelines and Adjuvant Online for early breast cancer. *Eur J Cancer* 2010; 46: 1382-1391. (PMID:20359886) [\[CrossRef\]](#)

44. Özmen V, Atasoy A, Gökmen E, Özdoğan M, Güler N, Uras C, Ok E, Demircan O, Işıkdogan A, Cabioglu N, Şen F, Saip P. Impact of Oncotype DX Recurrence Score on Treatment Decisions: Results of a Prospective Multicenter Study in Turkey. *Cureus* 2016; 8: 522. (PMID: 27081583)
45. Özmen V, Atasoy A, Gökmen E, Özdoğan M, Güler N, Uras C, Ok E, Demircan O, Işıkdogan A, Cabioglu N, Şen F, Saip P. Correlations Between Oncotype DX Recurrence Score and Classic Risk Factors in Early Breast Cancer: Results of A Prospective Multicenter Study in Turkey. *J Breast Health* 2016; 12: 107-111. (PMID: 28331745) [\[CrossRef\]](#)
46. Ozmen V, Gökmen E, Atasoy A, Özdoğan M, Güler N, Uras C, Ok E, Demircan O, Işıkdogan A, Saip P. Cost effectiveness of Oncotype DX test in patients with early-stage breast cancer in a middle-income country, Turkey: results of a prospective multicenter study. *The Breast* 2017; 32: 84-85. [\[CrossRef\]](#)
47. Tiberi D, Masucci L, Shedid D, Roy I, Vu T, Patocskaï E, Robidoux A, Wong P. Limitations of Personalized Medicine and Gene Assays for Breast Cancer. *Cureus* 2017; 9: 1100. (PMID: 28428927) [\[CrossRef\]](#)