



Systematic or Meta-analysis Studies

Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe

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ABSTRACT

Gene expression profiles with prognostic capacities have shown good performance in multiple clinical trials. However, with multiple assays available and numerous types of validation studies performed, the added value for daily clinical practice is still unclear. In Europe, the MammaPrint, OncotypeDX, PAM50/Prosigna and Endopredict assays are commercially available. In this systematic review, we aim to assess these assays on four important criteria: Assay development and methodology, clinical validation, clinical utility and economic value.

We performed a literature search covering PubMed, Embase, Web of Science and Cochrane, for studies related to one or more of the four selected assays.

We identified 147 papers for inclusion in this review. MammaPrint and OncotypeDX both have evidence available, including level IA clinical trial results for both assays. Both assays provide prognostic information. Predictive value has only been shown for OncotypeDX. In the clinical utility studies, a higher reduction in chemotherapy was achieved by OncotypeDX, although the number of available studies differ considerably between tests. On average, economic evaluations estimate that genomic testing results in a moderate increase in total costs, but that these costs are acceptable in relation to the expected improved patient outcome. PAM50/prosigna and EndoPredict showed comparable prognostic capacities, but with less economical and clinical utility studies. Furthermore, for these assays no level IA trial data are available yet.

In summary, all assays have shown excellent prognostic capacities. The differences in the quantity and quality of evidence are discussed. Future studies shall focus on the selection of appropriate subgroups for testing and long-term outcome of validation trials, in order to determine the place of these assays in daily clinical practice.

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Introduction

In the past decades, there has been a steady increase in the survival rates of patients with breast cancer. Among other factors like early screening and awareness, the majority of this effect is attributed to the concept of adjuvant therapy [1,2]. However, among all patients receiving adjuvant chemotherapy, the majority would not have developed metastases even without adjuvant therapy, whereas in contrast some patients without the indication for adjuvant therapy still develop distant metastases. A recent progress in

this optimal selection is the development of genomic profiling assays [3]. We chose four crucial criteria for determining the value of these assays.

Assay development and methodology

The first criterion is the methodological robustness, both during development and during the commercial activities. For example, the tests should be validated in a cohort independent from the training cohort, and should not be used in a patient population in which the test was not validated unless re-validation is performed. Furthermore, there should be little to no inter-test variation when the same tissue samples are tested multiple times.

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Another aspect of assay development is determining the target population. Therefore, studies need to focus on identifying subgroups which do not benefit from genomic testing since the outcome of the test overlaps with the stratification by the clinicopathological factors (e.g. when all or almost all triple-negative breast cancers are considered high-risk by the test).

Clinical validation

A second important factor is the effect on clinical outcome between the different test-outcome groups. Similar to classical biomarkers, a distinction can be made between the prognostic and the predictive value of a test [4]. Since the utility of genomic testing is in particular aimed at guiding decisions regarding chemotherapy, a predictive test, able to predict which patients will benefit from chemotherapy or not, is more valuable than a solely prognostic test which is only associated with the patient prognosis.

Clinical utility

The third criterion is the clinical utility of the test. Applying the test should lead to a shift in the indication of chemotherapy as compared to indication based on traditional parameters. In other words, if the patients using chemotherapy based on the test results are exactly the same patients as the ones using chemotherapy based on the traditional clinicopathological parameters, the test has no additional value.

Economic value

The fourth, and last criterion for genomic testing is the economic value of the test. Due to the commercialisation of the assays, the tests are more expensive than the regular pathological assessment, with costs ranging from €1800 to €3700 per test. In an era of emphasis on healthcare efficiency, the costs of the test should be justified by its clinical and health benefits, and the reduction in costs by reducing adjuvant therapy use.

Test descriptions

The first test, which was first developed in 2002 by van 't Veer et al. and for which the prognostic capacities were shown simultaneously by van de Vijver et al., is the 70-gene prognosis profile, better known as MammaPrint (Agendia BV, Amsterdam, The Netherlands) [5,6]. This assay uses the mRNA expression of 70 genes using microarray technology, to categorize patients in either a low or high risk. These 70 genes were identified from a total of 25,000 genes using supervised clustering.

The second test in this review is the 21-gene Recurrence Score, also known as the OncotypeDX Recurrence Score (RS) (Genomic Health Inc., Redwood City, CA). The test is based on the expression of 21 genes in FFPE cancer tissue, determined using reverse transcriptase PCR (RT-PCR) [7]. Of these genes, 16 genes are cancer-related and were selected out of 250 rationally selected candidate genes based on their prognostic capacity and consistency in test performances [7]. Based on these relative expressions, the Recurrence Score is calculated ranging from 0 to 100, with low risk ranging from 0 to 17, intermediate risk ranging from 18 to 30, and high risk ranging from 31 to 100. However, for the most important validation trial of this test, the risk categories in this trial were adjusted to 0–10, 11–25 and 26–100 for the low-, intermediate- and high risk respectively [8].

The third test included in this review is the Prosigna, based on the better-known PAM50 test (NanoString Technologies, Seattle,

WA). This test, based on the expression of 46 genes using quantitative PCR (qPCR) is able to distinguish between the molecular subtypes of breast cancer (luminal A, luminal B, HER2-enriched, normal-like and basal-like) [9]. Furthermore, it provides the risk of recurrence score (ROR) and the subsequent risk category. The test was adapted by NanoString in order to allow the use in local pathology laboratories [10].

The fourth and last test which will be discussed in this systematic review is the EndoPredict (Myriad Genetics Inc, Salt Lake City, UT). This assay uses the expression of 8 cancer-related and 3 reference genes determined by RT-PCR, which results in a risk score from 0 to 15 (EP), which is subsequently divided into low and high risk [11]. A special feature of the EndoPredict is the integration of tumor size and nodal status, resulting in an EP clinical score (EPclin). The EndoPredict can be performed in local laboratories, in contrast to the MammaPrint and OncotypeDX which are centrally determined and therefore need more elaborate logistical planning.

In this review, we evaluate four genomic assays available in Europe using a systematic evaluation focusing on all four major criteria with the aim to assess each test individually for its strengths and weaknesses.

Methods

Search strategy

This systematic review was to comprehensively cover all four aspects of the four commercially available genomic profiling tests in Europe on four different aspects: developmental and methodological robustness, extend of clinical validation, clinical utility and economic value. These items were chosen after a consensus meeting and cover those evaluation criteria we deemed most important. We searched PubMed, Embase, Web of Science and Cochrane for articles published before April 2016. The search strategy (supplementary document 1) was applied on April 7th 2016, and after evaluation of all abstracts it was updated at September 9th 2016. Abstracts were screened for relevance based on the title and abstract, and remaining full-text articles were screened based on the inclusion criteria.

Selection criteria

Articles were selected if they studied one of the four tests available in Europe: OncotypeDX, MammaPrint, Prosigna or Endopredict. Furthermore, the article should be original peer-reviewed research; abstracts, posters, reviews and meta-analyses were excluded. The article needed to cover one of the four criteria: development of the test, clinical validation, clinical utility or an economical evaluation. For the clinical validation studies, survival analysis was required, evaluating either the differences in survival between test-outcome groups, or the benefit of therapy in one or more test-outcome groups. For the clinical utility studies, decision impact studies were to be available in a representative cohort, and had to report both the absolute increase or decrease in chemotherapy as well as the shift from one treatment category to the other. Retrospective large-scale population-based impact studies were also included, reporting real-life shifts in the use of genomic testing and the subsequent changes in therapy decisions. Two reviewers (EJB, EB) independently selected articles that met the above inclusion criteria based on title and abstracts. Next, full-texts of potentially relevant articles were screened. Agreement concerning eligibility was achieved during consensus.

Data extraction and statistics

Data extraction was independently performed by the two reviewers. Data was collected concerning the performed test, the number of included patients, the results of the test, and survival outcome or change in treatment where appropriate. Disagreements in data extraction and interpretation were resolved during a consensus meeting. There were no changes in eligibility criteria during the selection of articles. All studies that fulfilled the inclusion criteria were included, independent of their methodological quality; no risk of bias assessment was performed. Both retrospective and prospective studies were included without exclusion of particular study designs with an emphasis on prospective RCTs (where available). Data were recorded in the tables as mentioned in the articles, no additional statistics were performed. Both point estimates and 95%CI were recorded, where appropriate and mentioned in the selected articles.

Due to the heterogeneity of the studies chosen, the patient selection and endpoints reported, no further statistical analyses could be performed. Results were stratified in (1) one of the four tests and (2) lymph node positive or lymph node negative patients or articles where the distinction could not be made or both groups were included.

For the clinical utility, extracted data from decision-impact studies were pooled (weighted by the number of patients) to give an estimate of the chemo-reduction and shift in therapy a test can establish. We only considered a change in chemotherapy and recorded the percentage of patients who would receive chemotherapy before the test, and after the test (as mentioned in the included articles). For the table on clinical validation, the number of patients who were high or low risk according to the test were recorded and the outcome in the groups. Outcomes were recorded as mentioned in the articles: distant metastasis or distant recurrence free survival, breast cancer specific survival, and overall survival were most frequently reported. Where known, both the point estimate and the 95%CI were recorded. The Hazard Ratio and corresponding 95%CI for the difference in outcome between the risk groups was recorded if this was mentioned in the articles. For the economic review, original evaluations were included if they compared costs beyond the assay costs alone. Evaluations could be cost minimization analyses (CMA), cost effectiveness analyses (CEA, comparing costs to life years) or cost utility analyses (CUA, comparing costs to quality-adjusted life years (QALYs)). To aggregate, QALYs were imputed for CMAs and CEAs (as predicted by the average and the life year gain, respectively) and costs were updated to Euros at price level 2016. When more than one (non-) genomic strategy was included in an economic evaluation, the (non-) genomic strategy with the highest QALYs was used in the review.

Results

Using our search strategy, we identified 1345 unique titles and abstracts. Limiting ourselves to the manuscripts only related to the topics of this review, we selected 280 studies for further full-text evaluation. From these 280 full-text manuscripts, we selected 149 papers for inclusion in this review: 11 about developmental validation, 12 about biomarker prediction, 50 about clinical studies, 28 about clinical utility and the effect on chemotherapy reduction, 44 economic evaluations and 4 studies making direct head-to-head comparisons on test outcome between two or more of the included tests (Fig. 1).

Assay development and methodology

In the development of MammaPrint, multiple evolutions were necessary to allow high-throughput screening of FFPE tissue. Glas et al. first converted the original research-based micro-array containing approximately 25,000 probes to a mini-assay with good concordance and reproducibility [12,13].

A second step was the conversion from frozen to FFPE tissue by Mittempergher et al., with an R^2 of 0.94 [14]. After this proof of principle, Sapino et al. further developed the MammaPrint towards an FFPE platform, again with a good correlation between FFPE and frozen tissue ($r = 0.92$), and a high concordance between high- and low-risk classifications between both methods (κ -score 0.82) [15]. Beuner et al. validated both the conversion to a mini-assay and the conversion from frozen tissue to FFPE retrospectively, by comparing the scores of both methods [16].

Gyanchandani et al. studied whether intratumoral heterogeneity might influence the outcome of a gene expression test in 74 ER-positive cases using most included gene expression panels, by assessing different tumor regions from the same FFPE block [17]. They showed that genomic assays with a higher number of included genes resulted in a lower rate of discordant samples. Drury et al. studied the use of 0.6 mm cores and compared these with full sections, to establish whether tissue-microarrays (TMAs) could be used for genomic profiling using OncotypeDX [18]. Although the total RNA yield was lower from tissue cores compared to full sections, the OncotypeDX Recurrence Score results from individual cores clustered closely, and had an excellent correlation with full-section RS (Spearman $R = 0.91$).

For the Endopredict, the use of pre-surgery biopsies and surgical sections from 40 ER-positive HER2-negative tumours was compared. It was shown that comparing both results resulted in a Pearson correlation coefficient of 0.92, showing that core needle biopsies can be used for genomic profiling using Endopredict [19]. Another aspect of the EndoPredict is decentral assessment, meaning that every individual pathological laboratory can perform this test and thereby reducing the logistical strain on the testing procedure. Denkert et al. tested this decentral evaluation [20]. The Pearson correlation coefficient for all measurements was a near-perfect 0.994, and 100% of the samples were assigned to the same EP risk group as the reference test. Furthermore, Kronenwett et al. showed that this decentral approach had excellent precision and reproducibility, although with a small sample size [21].

Although these published studies showed a good reliability and reproducibility, the MINDACT trial shows that there can be problems which hamper the reliability and feasibility of a test. Between May 2009 and January 2010, 162 patients were falsely identified as being high risk, due to a change in RNA-extraction solution [22]. Furthermore, of all 11,288 screened patients, there was a screening failure in 1182 patients (10%) in which the MammaPrint was not feasible [22].

Another concern for the reliability of test results is the ratio between tumor and normal tissue in the tested specimen. Elloumi et al. showed that an increase of normal tissue in the specimen leads to biased test results when compared to uncontaminated tumor tissue test results [23]. For the PAM50 this bias was linear, showing a more favourable outcome with increasing normal tissue content. For the MammaPrint and OncotypeDX the bias was unpredictable, switching both from low to high risk and vice versa with increasing normal tissue content. All tests have since developed strategies to mitigate this bias.

A couple of studies directly compared the test results of multiple tests performed on one tumor. In the OPTIMA Prelim trial, patients were randomized between standard therapy or OncotypeDX-directed therapy [24,25]. Among others, also Mam-

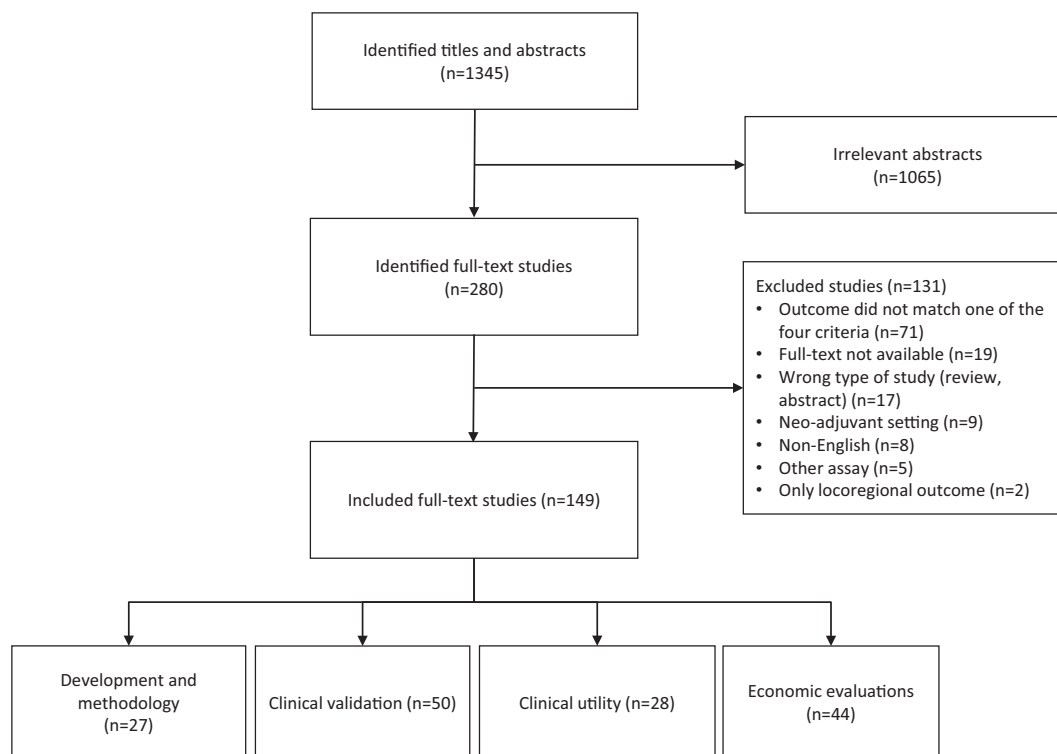


Fig. 1. A diagram showing the inclusion of relevant papers in the systematic review.

maPrint and Prosigna tests were performed. Strikingly, the kappa measurements were between 0.40 and 0.53. In the same cohort of patients, OncotypeDX predicted 17.9% to be high risk, compared to 38.6% and 34.5% for MammaPrint and Prosigna respectively. This pilot trial is now followed by the OPTIMA trial, in which treatment directed by the Prosigna assay is compared with regular care. In a smaller prospective study, 52 samples were analysed with both the OncotypeDX and Prosigna, showing a Spearman correlation coefficient of 0.08 [26]. Remarkably, 57.1% of the patients classified as high risk by Prosigna were classified as low risk by OncotypeDX. In a similar study comparing Endopredict and OncotypeDX results in 34 samples, a Pearson correlation of 0.65 was shown, with a concordance between risk categories of 76% [27].

Prediction of test results

Theoretically, a genomic profile can have an excellent prognostic value, but is 100% predicted by the occurrence of other markers and therefore has no added value. Therefore, it is crucial to establish the added value of the test, by testing whether the test result can be predicted by standard clinicopathological parameters. This testing could identify subgroups for which the test is not valuable. We identified 12 studies evaluating this effect, which are reported in Table 1. In general, tumours which are (a combination of) grade 1, PR-positive and/or have a Ki-67 expression lower than 10%, are almost always low risk when genomic testing is performed. Similarly, tumours which are (a combination of) grade 3, PR-negative and/or have a Ki-67 score of more than 40%, are almost always high-risk. For these subgroups, genomic profiling provides little additional information.

Clinical validation

A total of 50 studies was identified assessing the clinical benefit of the genomic assays; 21 assessing the MammaPrint, 20 assessing the OncotypeDX, 5 assessing the PAM50/Prosigna and

4 assessing the Endopredict. Most of the studies were retrospectively stratifying the cohort in separate risk categories determined by the test, and showing a difference in either distant metastasis-free, disease-free or overall survival. Table 2 shows the results of the retrospective included studies, according to test and patient inclusion. In general, the studies are difficult to compare due to different patient inclusion and outcome measures. All published studies showed a good differentiation in high and low risk and were associated with survival (both Distant Metastases/Recurrence Free Survival (DMFS/DRFS) as Overall Survival (OS)). In more detail, MammaPrint was reported to be of significant prognostic value for patients with lymph node negative breast cancer and the results of the test correlated well with Adjuvant!, St Gallen and NIH guidelines and the NPI. For lymph node positive disease, the hazard ratios for DMFS and Breast Cancer Specific Survival (BCSS) showed a significant difference in prognosis for low versus high risk according to MammaPrint. In the remaining articles (without specific classification or LN- and LN+ combined) the MammaPrint was also of prognostic value; most of the results showed a significant difference in outcome between low and high risk.

With respect to OncotypeDX, most of the studies in patients with LN negative disease studied the DRFS and showed a significant difference in outcome between low, intermediate and high risk patients. Paik et al. showed a statistical different effect of chemotherapy in the three risk groups with a significant interaction term between chemotherapy and the Recurrence Score. One case-control study showed a significant difference between both groups. Besides, the study in LN+ disease also showed a significant interaction between the RS and clinical benefit of chemotherapy for the first 5 years after treatment. The remaining studies (combined LN- and LN+ and one study in patients with metastatic disease) showed a good discrimination between the three risk groups and a significant difference in outcome in most of the studies.

Studies that used the PAM50 showed a good discrimination, and a significant interaction between treatment and outcome in

Table 1
Marker prediction, according to test and nodal status.

Marker prediction					
Authors	Year	Patients (N)	Markers in best-fit model	R ² best fit model	Subgroups little/no benefit of testing (>75% in risk category)
MammaPrint					
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>					
Cardoso [22]	2016		NA	NA	Grade 1 93% low risk Grade 3 75% high risk ER- PR- 96% high risk
Gevensleben [28]	2010	140	NA	NA	St. Gallen high risk 80% high risk St. Gallen low risk 86% low risk Grade 1 79% low risk Grade 3 76% high risk PR-negative 76% high risk ^a
OncotypeDX					
<i>Lymph node negative</i>					
Chaudhary [29]	2016	350	NA	NA	PR+ 95% low or intermediate risk
Dialani [30]	2016	319	ER, PR, HER2, tumor grade	0.55	NA NA
Sparano [8]	2015	8523	NA	NA	PR- 5% low risk Grade 3 11% low risk
Ingoldsby [31]	2013	52	PR (allred), nuclear pleomorphism (np), survivin	NA	Grade 1 100% low or intermediate risk PR <2, np-score 3 100% high risk
Sahebjam [32]	2011	53	PR, Ki-67	0.84	Ki-67 <10% 100% = low or intermediate risk
Auerbach [33]	2010	138	Mitotic count, PR	NA	PR+ & Mitotic count 1 or 2 100% = low or intermediate risk PR- & Mitotic count 2 or 3 75% = high risk (0% low risk)
Flanagan [34]	2008	42	ER, PR, grade, HER2, mitotic count	0.66	Grade 1 100% low or intermediate risk Grade 3 83.3% high risk (0% low risk)
Wolf [35]	2008	300	NA	NA	PR+ & Grade 1/2 94% low or intermediate risk
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>					
Gluz [36]	2016	2642	NA	NA	Grade 1 ~90% low or intermediate risk Ki-67 <20%, PR >20% ~95% low or intermediate risk Ki-67 >40% ~90% high risk
Bradshaw [37]	2013	158	ER (allred), PR (allred), Ki-67	0.62	NA NA
Allison [38]	2012	173	PR, tumor grade	Unknown (p < .001)	Grade 1 & PR >5 (allred) 100% low or intermediate risk Grade 3 & PR <5 (allred) 80% high risk (0% low risk)
Williams [39]	2011	133	NA	NA	Ki-67 <10% 99% = low or intermediate risk

^a Not designed to predict test results, but data are provided in the manuscript.

one study, this was however not confirmed in Liu et al. Three studies showed a significant association with distant recurrences. For studies that used EndoPredict differences between high and low risk were associated with outcome or showed a low proportion of distant metastases in the low risk group.

Both the PAM50/Prosigna and EndoPredict have a quality B level of evidence in all of their validation studies by performing them in established clinical trials, according to Simon et al. [83] For MammaPrint one level A trial is available [22], all other studies are level C quality or lower. For OncotypeDX, there is a mix of two level A trials [8,36], some level B studies showing predictive capacities of OncotypeDX, and level C/D studies in retrospective or case-control studies. All level A evidence will be discussed in the next paragraphs.

MINDACT

The MINDACT trial evaluated the use of the MammaPrint together with Adjuvant Online, an online tool using clinicopathological information for risk stratification [22]. Patients with discordant risks based on the clinical and genomic assessment, were randomized between chemotherapy or no chemotherapy. The primary study subgroup were the patients with a clinical high and genomic low risk tumor who were randomly allocated to receive no chemotherapy. The distant metastasis-free survival of this group was 94.7% at 5 years, which was significantly higher compared to a pre-determined null hypothesis of 92%. Therefore, it was concluded that the prognosis of these clinically high-risk, but genomic low risk patients without chemotherapy was good enough to justify the abstention of chemotherapy.

The trial is labelled as phase 3 RCT and the results are regarded as level IA evidence. However, the design of the primary analysis is that of a cohort study, since it only assessed the patients who had a discordant risk and did not receive chemotherapy. In a secondary per-protocol analysis, comparing the c-high/g-low patients with and without chemotherapy, a HR of around 0.65 was shown in favor of chemotherapy, which was significant for DFS (90.3% vs 93.3%, p = .026), but not for DMFS (94.8 vs 96.7, p = 0.106) or OS (97.3 vs 98.8, p = 0.245). In summary, although the prognosis of this clinically high-risk group is good without chemotherapy, it is significantly better when receiving chemotherapy.

Another secondary outcome is the effect of chemotherapy in patients who were clinically assessed as low-risk, but with a genomic high risk profile. In this subgroup, no statistically significant benefit of chemotherapy was observed for either DMFS (HR 0.90 95% CI 0.40–2.01), DFS (HR 0.74 95% CI 0.40–1.39) or OS (HR 0.72, 95% CI 0.23–2.24), indicating that a high-risk MammaPrint test result does not predict an effect of chemotherapy for these low-risk patients. Although this analysis is underpowered, and no formal interaction test was performed, the authors conclude that the MammaPrint failed to show its value as a predictive biomarker, not being capable of identifying patient who would benefit from chemotherapy.

TAILORx

The TAILORx trial was designed to assess the clinical use of OncotypeDX to decide on the chemotherapy administration, especially in the intermediate risk group. For this, 10,273 patients

Table 2
Clinical validation, according to test and patient inclusion.

Clinical validation							
Authors	Year	LOE	Patients (N)	Low/High risk (n)	Outcome	HR (95%CI)	Concordance
MammaPrint							
<i>Lymph node negative</i>							
Wittner [40]	2008	D	N = 100, LN-, postmenopausal	Low 27 High 73	NPV DM 100% (87–100) PPV DM 12% (6 –2 2)		
Van 't Veer [5]	2002	D	N = 78, LN-, <5cm, <55 years	Low 44 High 34	PPV DM 83%		
Mook [41]	2010	D	N = 148, T1-2N0, 55–70 years	Good prognosis 91 Poor prognosis 57	Validation set 2/19 incorrect DMFS 93% (SE 3%) vs 72% (SE 6%) BCSS 99% (SE 1%) vs 80% (SE 5%)	DMFS 4.6 (1.8–12.0; p = .001) BCSS 2.0 (1.0–4.0; p = .04)	Adjuvant! 62 (42%) good prognosis; 45 (30%) poor prognosis St. Gallen guidelines 29 (80.6%); 30 (83.4%) NIH guidelines; 23 (63.8%) Adjuvant! Online
Na [42]	2011	D	N = 36, cT1-2N0M0	Low 5 High 31	40% low-risk prognosis, 60% high-risk prognosis		
Drukker [43]	2013	C	N = 427, cT1-3N0M0	Low 219 High 208	5-yrs DRFS 97.0 5-yrs DRFS 91.7		
Bueno de-Mesquita [44]	2007	C	N = 427, cT1-4N0M0, <61 years	Good 219 Poor 208			Discordance Adjuvant! 160 (37), St Gallen 168 (39), NPI 117 (27)
Bueno de-Mesquita [45]	2009	D	N = 123, pT1-2N0, <55 years Second series N = 151	Good 52% Poor 48% Good 40% Poor 60%	DMFS 98(±2) good vs 78(±6) poor OS 97(±2) good vs 82(±5) poor DMFS 86(±5) vs 50(±6) OS 94(±3) vs 51(±5)	5.7 (1.6–20) 3.4 (1.2–9.6) 5.5 (2.5–12) 10.7 (3.9–30)	
<i>Lymph node positive</i>							
Knauer [46]	2010	D	N = 541 LN+, received ET or ET +CT	Low 252 High 289	BCSS 97% low vs 87% high DMFS 95% low vs 82% BCSS low: 97% ET, 99% ET+CT BCSS high: 81% ET, 94% ET+CT DDFS low: 93% ET, 99% ET+CT DDFS high: 76% ET, 88% ET+CT OS 97% vs. 76% high risk DMFS 87% low vs 63% high (p < .01)	4.81 (1.98–11.67) 3.88 (1.99–7.58) 0.58 (0.07–4.98) 0.21 (0.07–0.59) 0.26 (0.03–2.02) 0.35 (0.17–0.71) HR 2.211, p = .005	
Saghatchian [47]	2012	D	N = 173, 4–9 positive lymph nodes	Low 70 High 103			
Mook [48]	2009	D	N = 241, T1-3, 1–3 positive LN	Good 99 Poor 142	DMFS 91% (SE 4%) vs 76% (SE 4%) BCSS 96% (SE 2%) vs 76% (SE 4%)	4.13 (1.72–9.96; p = .002) 5.70 (2.01–16.23; p = .001)	Discordant Adjuvant! 77 (32%)
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>							
Mook [49]	2010	D	N = 964, pT1	Good 525 Poor 439	DMFS 87% SE 2% vs 72% SE 3%, BCSS 91% SE 2% vs 72% SE 3%	DMFS 2.70 (1.88–3.88) BCSS 4.22 (2.70–6.60)	
Van de Vijver [6]	2002	D	N = 295, stage I-II, <53 years	Poor 180 Good 115	Mean OS 54.6(±4.4) vs 94.5(±2.6) DMFS 85.2(±4.3) vs 50.6(±4.5)	5.1 (2.9–9.0)	
Knauer [50]	2010	D	N = 168, T1-3N0-1, HER2+	Good 20 Poor 69	DMFS 84% vs DMFS 55% No data BCSS	DMFS 4.5 (1.1–18.7) BCSS 3.8 (0.9–15.8)	
Drukker [51]	2014	D	N = 295, T1-2N0-1M0, <53 years	Low 115 High 180	25-yrs DMFS 60.4 (45.3–80.5) vs 41.6 (32.6–53.1) 25-yrs OS 57.3 (44.8–73.2) vs 39.7 (31.7–49.8)	DMFS 3.1 (2.02–4.86) OS 2.9 (1.90–4.28)	
Drukker [52]	2014	D	N = 1053, T1-3N0-1M0	Low 561 High 492	LRR 6.1 (4.1–8.5) LRR 12.6 (9.7–15.8)	2.40 (1.54–3.74)	
Cardoso [22]	2016	A	N = 6693 early stage BC	Low CR-low GR 2745, Low CR-High GR 592, High CR-Low GR 1550, High CR-High GR 1806	Chemo: 95.8 (92.9–97.6) No chemo: 95.0 (91.8–97.0) DMFS No chemo: 94.4 (92.3–95.9) DMFS chemo: 95.9 (94.0–97.2)	CT vs no CT: 1.17 (0.59–2.28) CT vs no CT: 0.78 (0.50–1.21)	14.3%

(continued on next page)

Table 2 (continued)

Clinical validation							
Authors	Year	LOE	Patients (N)	Low/High risk (n)	Outcome	HR (95%CI)	Concordance
Buyse [53]	2006	D	N = 307	Clinical low risk GLR 52/GHR 28 Clinical high risk GLR 59/GHR 163	10-yr OS 0.88/0.69 0.89/0.69	DMFS 2.32 (1.35–4.00) vs Adjuvant! 1.68 (0.92–3.07) OS: 2.79 (1.60–4.87) vs 1.67 (0.93–2.98)	
Kunz [54]	2011	D	N = 689, 35–55 years	Low 42% High 58%	10-yr OS: 90.2% (86.3–94.1) 65.2% (60.3–70.1) 10-yr DMFS 87.7 (83.6–91.8) vs 64.5 (59.8–69.2)		St. gallen low risk 4, high risk 6, intermediate 34
Kok [55]	2012	D	121 with TAM, 151 no TAM, 92 TAM for M1	Good 83/Poor 38 Good 85/Poor 66 Good 45/Poor 47	BCSS 80.6 (SE 4.7) vs 63.4 (SE 8.2) BCSS 90.2 (3.3) vs 63.3 (6.3) Median TTP 20.9 vs 6.6 months	2.78 (1.30–5.94) 4.52 (2.01–10.2) 2.55 (1.59–4.07)	
Ahn [56]	2013	C	N = 82, ER+, with intermediate score Oncotype	Good 66 Poor 16		Multivariable 10.19 (1.05–99.01); P = .013	
Ishitobi [57]	2010	D	N = 102, <70 yrs,	Low 20 High 82	DMFS 100% DMFS 94%		PPV 9.8%, NPV 100%
OncotypeDX							
<i>Lymph node negative</i>							
Toi [58]	2010	D	N = 200, N0, ER+	Low 48% Intermediate 20% High 33%	DRFS 3.3 (1.1–10.0) vs 0% vs 24.8 (15.7–37.8) OS 6.4 (2.9–13.6) vs 2.6 (0.4–16.8) vs 19.1 (11.3–31.3)	HR for 50-point increase 6.09 (2.17–16.7), p < .001	
Paik [7]	2004	D	N = 668, N0, ER+	Low 51% Intermediate 22% High 27%	DRFS 6.8 (4.0–9.6) vs 14.3 (8.3–20.3) vs 30.5 (23.6–37.4)	3.21 (2.23–4.61); p < .001	
Naoi [59]	2013	D	N = 459, N0, ER+	Low 286 Intermediate 81 High 92	RFS better low vs intermediate p = .0014 and high P = 1.7e-11		
Sparano [8]	2015	A	N = 10253, N0, HR +, HER2-, 1.1–5.0 cm	Low 1629 Intermediate 6907 High 1736	RS 0–10: 5-yr invasive DFS 93.8 (92.4–94.9); DRFS 99.3 (98.7–99.6); RFS 98.7 (97.9–99.2); OS 98.0 (97.1–98.6)	HR 2.16 for 50 units in RS (1.26–3.68; P = .007)	
Mamounas [60]	2010	B	N = 895, N0, ER+	Low 862 Intermediate 368 High 444	10-yr LRR 4.3 (2.3–6.3) low, 7.2 (3.4–11.0) intermediate, 15.8 (10.4–21.2) high RS Placebo: p = .022; 10.8% (5.8–15.8%) vs 20.0% (9.9–30.0%) vs 18.4% (9.5–27.4%) CT+TAM: p = .028; 1.6% (0–3.5%) vs 2.7% (0–6.4%) vs 7.8% (2.6–13%)		
Sgroi [61]	2013	B	N = 665, N0, ER+	BCI vs OncotypeDX vs IHC4	-Early DR: BCI HR 2.77 [95%CI1.63–4.70], LR- $\Delta\chi^2=15.42$, p < 0.0001; 21-gene recurrence score HR 1.80 [1.42–2.29], LR- $\Delta\chi^2=18.48$, p < .0001; IHC4 HR 2.90 [2.01–4.18], LR- $\Delta\chi^2=29.14$, p < .0001 -Late DR BCI HR 1.95 [95% CI 1.22–3.14], LR- $\Delta\chi^2=7.97$, p = .0048; 21-gene recurrence score HR 1.13 [0.82–1.56], LR- $\Delta\chi^2=0.48$, p = .47; IHC4 HR 1.30 [0.88–1.94], LR- $\Delta\chi^2=1.59$, p = .20		
Paik [62]	2006	B	N = 651, N0, ER+	Low 353 Intermediate 134 High 164	DR: Low chemotherapy RR 1.31 (0.46–3.78), intermediate RR 0.61 (0.24–1.59); High RR 0.26 (0.13–0.53)	Test interaction chemotherapy and RS p = .038	
Tang [63]	2011	B	N = 1444 N0, ER+	Intermediate RSPC (17.8%)	DR vs RS RSPC vs RS: intermediate 17.8 vs 26.7, and low risk 63.8 vs 54.2	Interaction term RSPC chemotherapy p = .10	
Yorozuya [64]	2010	D	N = 40, N0, ER+, Stage I-IIA	Cases 10, controls 30. Cases: low 3, intermediate 1, high 6; Controls low 19, interm 8, high 3	Mean RS cases 40.0 (21.1–58.9), controls 17.8 (13.8–21.9); p < .001		
<i>Lymph node positive</i>							
Albain [65]	2010	B	N = 367, N+, ER+, postmenopausal	Low 146 Intermediate 103 High 118	DFS TAM alone HR 2.64 (1.33–5.27) 50point difference. Benefit chemotherapy low risk: HR 0.97 (0.54–1.93), high risk HR 0.59 (0.35–1.01)	Interaction RS treatment p = .029 1 st 5 yrs, beyond 5 yrs p = .58	

Table 2 (continued)

Clinical validation							
Authors	Year	LOE	Patients (N)	Low/High risk (n)	Outcome	HR (95%CI)	Concordance
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>							
Gluz [36]	2016	A	N = 348, pN0-1, HR+	Low risk 18.1% Intermediate 60.4% High 21.6%	3-yrs DFS 98% RS<=11, 96% RS 12-25, 92% RS > 25		
Dowsett [66]	2010	B	N = 1231, HR+, postmenopausal	N0: 59, 26, 15% N+: 52, 31, 17	9-yr DR 4%, 12% and 25% in N0 9-yr DR 17%, 28% and 49% in N+	RS 50 units: N0: 5.25(2.84-9.73), N+3.47 (1.64-7.38)	
Cuzick [67]	2011	B	N = 1125, ER+		DR RS vs IHC4 score (r = 0.68 for TTDR, N0; r = 0.71 for time to recurrence [TTR], all; r = 0.69 TTR,N0)		r = 0.72
Goldstein [68]	2008	D	N = 465, HR+, 0-3 positive nodes	Low 46% Intermediate 30% High: 24%	5-yr recurrence rate low risk: 0-1 positive nodes 3.3 (2.2-5.0), 2-3positive nodes: 7.9% (4.3-14.1)	Multivariable - 50-point increase) 2.12 (0.97-1.65)	
Le Du [69]	2015	D	N = 1030, stage I, ER+, HER2-	Low 571 Intermediate 370 High 89	DDFS: RS predictor DDFS p < .001. High RS 76.4% (59.2-87.1), low RS 95.9%(93.0-97.6)	HR 2.197 (0.901-5.356), p = .083	
Tang [70]	2010	B	N = 668	Low: 338 Intermediate 149 High 181	Adjuvant! Low: DRFS 5.6 low, 10% intermediate risk, 18.2% high. Adjuvant! Intermediate: 13.4, 13.9, 43.2. Adjuvant! High: 5, 23.4, 31.5%	CT interaction P = .031 DRFS, P = .011 OS, P = .082 DFS,	Concordance with Adjuvant! 0.49, Adjuvant! OS interaction CT p = .009, DRFS p = .219 Kappa Adjuvant! 0.091, Adjuvant! Transbig 0.182, NCCN 0.091 Correlation RS - PR p = .006, with G3 p = .002, low Ki67 p < .001 RS and NPI r = -0.0737, p = .4527
Freitas & Simon [71]	2011	D	N = 22, ER+, HER-early stage	Low 11, Inter/high 11			
Aktas [72]	2013	D	N = 68, HER2-	Low 30 Intermediate 29 High 9			
Acs [73]	2013	D	N = 106, low grade, ER+	Low 68 Intermediate 38 High 0	Comparison with mammostrat (immunohistochemistry)		
Kok [74]	2009	D	N = 246, M+, TAM treated	Low-intermediate 28 High 41	78-gene TAM response, Oncotype DX and HOXB13-IL17BRratio-TTP:HR 2.2 (1.3-3.7, P = .005), 2.3 (1.3-4.0, P = .003) & 4.2 (1.4-12.3, P = .009)	Multivariable model 1.94 (1.01-3.73); p = .048	Concordance 45-61%
PAM50/Prosigna							
Martin [75]	2013	B	N = 820		OS low PAM50 HR 0.23 (0.09-0.57), p < .001	Interaction PAM50-treatment: p = .006 cont. p = .019 groups	
Liu [76]	2015	B	N = 1094, <=60 yrs, N+/high risk N0	Low 3.4% Intermediate 17.9%, high 78.7%	Higher ROR worse RFS p = .03. Multivariable ROR high vs low/int HR 1.98 (0.53-7.45; p = .311)	Subtypes p = .002 multivariable model. Not predictive treatment effect p-interaction = 0.23	
Sestak [77]	2015	B	N = 2137, HR+, postmenopausal		DR High risk: 16.6 (13.1-20.9), intermediate 8.3 (6.1-11.2), low 2.4 (1.6-3.5). HR high 6.9 (4.54-10.47), intermediate HR 3.26 (2.07-5.13) compared to low	Added to clinical factors: Univariable LRchi2 = 67.94; Multivariable LRchi2 = 35.25	r = 0.36
Gnant [78]	2014	B	N = 1478, ER+, postmenopausal	Low 502 Intermediate 478 High 498	DR ROR HR 1.03 (1.02-1.04, P < .0001); log-likelihood test: ΔLRχ2 = 53.49; P < .0001 DRFS 10-yrs low risk 96.7 (94.6-98.0, intermediate 91.3% (88.1-93.8), high 79.9% (75.7-83.4)		Spearman's correlation coefficient: 0.32, P < .0001
Filipits [79]	2014	B	N = 1246	Low 460 Intermediate 416 High 370	Late DRFS compared to clinical factors: DLRC2 15.32, P < .001. 15-yrs DRFS low 97.6 (94.7-98.9), intermediate 90.9 (85.9-94.2), high 82.5 (74.8-88.1)		

(continued on next page)

Table 2 (continued)

Clinical validation		Year	LOE	Patients (N)	Low/High risk (n)	Outcome	HR (95%CI)	Concordance
EndoPredict								
Fitzal [80]		2015	B	N = 1324, ER+, HER2-, Postmenopausal	High: 683 Low: 641	10-year LRFS: 91% 10-year LRFS: 97.5%	HR 1.31 (1.16–1.48), p < .005	58–61% high/int according to clinical guidelines to low risk
Dubsky [81]		2013	B	N = 1702, ER+, HER2-, early stage, Postmenopausal	High 37% Low 63%	Low: 10-year DM 95.3% (93.4–97.3)		C index with clinic-pathological parameters: 0.716
Dubsky [82]		2013 (B/C)	B	N = 1702, ER+, HER2-, Postmenopausal	High 51% Low 49%	Low: 1.8% DM 10 yrs	0–5 yrs: 1.20 (1.10–1.31) >5 yrs: 1.28 (1.10–1.48)	
Filipits [11]		2011	B	N = 378+1324		DR EPclin recurrence rates 4% and 4% in EPclin low-risk, 28% and 22% in EPclin high-risk (P < .001) and ABCSG-8 (P < .001), respectively.	Multivariable model 1.19 (1.04–1.36) and 1.26 (1.15–1.38)	

LOE = level of evidence according to Simon and Hayes [83], LN+ = Lymph Node positive, LRFS = local recurrence free survival, BC = breast cancer, DM = distant metastases, CR = clinical risk, GR = Genomic Risk, TTP = Time to Tumour Progression, LRR = loco-regional recurrence, BCI = breast cancer index assay, TTP = time to progression, NPI = Nottingham prognostic index.

were enrolled, who all had ER- and/or PR-positive, node-negative disease but did have an indication for chemotherapy based on the NCCN-guidelines. Low-risk patients (based on Recurrence Score) received endocrine therapy only; high risk patients received both endocrine and chemotherapy. Intermediate risk-group patients were randomly allocated to either endocrine therapy alone or a combination of endocrine and chemotherapy. Until now, only the results of the low-risk patients were published [8].

A total number of 1626 patients with a low-risk OncotypeDX test received no chemotherapy. The rate of DFS at 5 years was 93.8%, the freedom from distant recurrence was 99.3% and the overall survival was 98%. Similar to the MINDACT trial, this shows that genomic testing can identify patients with a good prognosis without chemotherapy, despite a clinical indication for chemotherapy.

In a similarly designed trial (RxPonder), node-positive patients with HR+ breast cancer and a low or intermediate test result are randomly assigned to hormone therapy with or without chemotherapy [84]. Results of this trial will show whether it is safe to withhold chemotherapy based on a low or intermediate test result population despite the high-risk nodal status.

WSG PlanB

In the West German Study Group Phase III PlanB Trial, 3198 clinically high-risk patients were enrolled, including 41.1% with node-positive disease. Although originally designed to compare two regimes of chemotherapy, after inclusion of 274 patients the study was amended to omit chemotherapy in patients with a low-risk OncotypeDX test result, despite their high clinical risk [36].

In this high-risk population, 348 patients received no chemotherapy based on a low-risk Recurrence Score of <12. At 3 years of follow-up, the disease-free survival was 98.4% in this subgroup, indicating again that genomic subtyping can identify a clinically high-risk subgroup with an excellent prognosis without chemotherapy, although longer follow-up is warranted for definite conclusions. Similar to the TAILORx, this study used an alternative cut-off for low-risk scores, which needs to be considered when interpreting the results.

Clinical utility

A total of 28 studies which evaluated the clinical utility of assays has been identified, of which 22 for OncotypeDX, four for MammaPrint, and one for both Prosigna and Endopredict. Almost all studies compared the (hypothetical) application of chemotherapy for the same patient, with and without the results of the genomic test. In general, de-escalation from chemotherapy to no therapy or endocrine therapy alone was higher than the escalation towards chemotherapy, which led to a decrease in chemotherapy use for all tests. When the results were pooled per assay, the decrease in chemotherapy was the most pronounced for OncotypeDX (45.7% from chemotherapy to endocrine therapy alone or no adjuvant therapy) compared to MammaPrint (32.2% decrease) (Table 3). However, these pooled results should be interpreted carefully, since there is a large difference in the number of studies per test, the baseline patient populations and study designs.

For OncotypeDX, three other studies evaluated the use of chemotherapy in population studies [113–115]. Two of them observed a decrease in chemotherapy use during the designated years, and an increase in genomic testing [114,115]. However, no direct relation was observed between both results. In the study of Su et al., performed in a US medicare population between

Table 3
Clinical utility, according to test and nodal status.

Clinical utility						
Authors	Year	Patients (N)	% chemotherapy before test	% chemotherapy after test	% change to chemotherapy	% change to HT/no therapy
MammaPrint						
<i>Lymph node negative</i>						
Drukker [85]	2014	N = 414, T1-3	49	37	4.3	29.1
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>						
Pohl [86]	2016	N = 107, HR+ HER2–	56.1	39.2	40	62
Exner [87]	2014	N = 75, grade 1 or 2, T 1-3cm, HR+ HER2–	41.3	33.3	9.1	32.3
Cusumano [88]	2014	N = 194, T1-3N0-1	60.8	60.8	34.6	22.3
<i>Subtotal MammaPrint</i>						
		N = 790	52.1	42.8	17.0	32.2
OncotypeDX						
<i>Lymph node negative</i>						
Ozmen [89]	2016	N = 165, T1-3N0-1mic, HR+ HER2–	55.8	37	13.7	44.6
Levine [90]	2016	N = 972, T1-4N0-1mic, HR+ HER2–	22*	20.7	10.9	62.6
Leung [91]	2016	N = 146, T1-3N0-1mic, HR+	52.1	37.7	4.3	31.6
Gligorov [92]	2015	N = 100, T1-3N0-1mic, HR+ HER2–	52	25	10.9	61.2
Lee [93]	2015	N = 212, T1-3N0-1mic, HR+	70.7	22.1	9.7	72.7
Jaafar [94]	2014	N = 47, T1-2N0, HR+ HER2–	48.9	25.5	4.2	52.2
Davidson [95]	2013	N = 150, T1-3N0, HR+ HER2–	41.3	31.3	17	48.4
Holt [96]	2013	N = 142, T1-3N0-1mic, HR+	40.1	30.3	14.1	45.6
Biroschak [97]	2013	N = 50, T1-3N0, HR+	72	70	28.6	13.9
Ademuyiwa [98]	2011	N = 276, T1-3N0 HR+ HER2–	45.3	32	22.5	56.8
Albanell [99]	2011	N = 107, T1-3N0, ER+ HER2–	37	27	17.6	56.4
Lo [100]	2010	N = 89, T1-2N0, HR+	47.2	25.9	6.5	47.6
Henry [101]	2009	N = 29, T1-3N0, HR+	45	28	13	54
Oratz [102]	2007	N = 74, T1-3N0, HR+	48	48	20	21.2
<i>Early stage breast cancer (combined LN- and LN+, other groups or not specified)</i>						
Kuchel [103]	2016	N = 137, T1-3N0-1, HR+ HER2–	50.4	27.7	18.2	62.3
Bargallo [104]	2014	N = 96, T1-3N0-1 ER+ HER2–	48	31	16	45.7
Yamauchi [105]	2014	N = 124, T1-3N0-1, HR+ HER2–	51	24	11.5	63.5
Fried [106]	2014	N = 111, T1-3N0-1, HR+	29.7	27.9	14.1	39.4
Cheung [107]	2014	N = 64, T1-2N0-1, HR+ HER2–	61	55	16	20.5
Eiermann [108]	2013	N = 366, T1-3N0-1, HR+ HER2–	57	46	25	38
De Boer [109]	2013	N = 151, T1-3N0-1, HR+ HER2–	44.4	37.1	15.5	35.8
Geffen [110]	2011	N = 135, T1-2N0-1	47	36	13.9	38.1
<i>Subtotal OncotypeDX</i>						
		N = 3743	50.2	30.6	14.6	51.1
PAM50/Prosigna						
Martin [111]	2015	N = 200, T1-2N0, HR+ HER2–	30%	28%	12.9%	37.3%
EndoPredict						
Muller [112]	2013	N = 167, T1-3N1-3, HR+ HER2–	63.8%	47.7%	34%	53.2%

* Not included in pooled data, since pre-test chemotherapy also included 34% unsure.

2008 and 2011, no difference in the use of chemotherapy was observed despite an increase of assay use from 9 to 17.2% [113].

Two other studies evaluated the use of chemotherapy between patients with and without genomic testing [116,117]. In the large study performed by Ray et al. (n = 7004), 22% of chemotherapy was observed in patients without testing, whereas 26% used chemotherapy after genomic profiling. In contrast, Stemmer et al. (n = 951) observed in a node-positive population, a 70% chemotherapy use without testing and a 24.5% chemotherapy use after genomic testing.

In a similar study design, Kuijer et al. observed a 10% lower rate of chemotherapy for patients with genomic testing using MammaPrint [118].

Economic value

Forty-four original economic evaluations were found, of which 32 on Oncotype DX, 7 on MammaPrint, 1 on EndoPredict and 4 direct comparisons between tests (Table 4). Most evaluations

compared genomic testing to a variety of strategies without genomic testing; four evaluations were head-to-head comparisons between genomic policies. Of the evaluations, 5 only estimated costs (CMAs), 1 estimated life years without QALYs (CEA) and 38 estimated QALYs (CUAs).

Methodologically, only 2 evaluations (both CMA) compared measured outcomes between two actual patient groups with and without genomic testing [113,119]. The remaining 42 evaluations all used mathematical (mostly Markov) modelling to compare estimated outcomes for different policies, for the same actual or hypothetical group of patients. These mathematical models typically estimated a decrease in chemotherapy (because the shift to low risk exceeds the shift to high risk), a decrease in recurrence (because the decrease in high risk exceeds the increase in low risk), and an increase in life years and QALYs (due to the decrease in recurrence and toxicity). Total health care costs may go up or down, depending on the balance between the assay costs and savings on chemotherapy and recurrence. Three studies also included savings on productivity [120–122].

Table 4
Economic evaluations, according to test and nodal status.

Authors	Year	Comparator	Patient group	Country	Impact on costs	Impact on QALYs	Impact on life years	Economic conclusion
MammaPrint compared to no genomic testing								
<i>Lymph node negative</i>								
Bonastre [124]	2014	Adjuvant! Online	NO	France	€ 2037	0.02	0.01	€ 134,000 per QALY
Chen [125]	2010	Adjuvant! Online	NO	US	\$ 1440	0.153	0.143	\$ 10,000 per QALY
Exner [87]	2014	Usual care	NO HR+ HER2–	NL	€ –3779	0.73	–	Dominant
Kondo [126]	2012	Best practice	NO ER+ HER2–	Japan	\$ 2571	0.06	0.048	\$ 43,044 per QALY
Retèl [127]	2010	Adjuvant! Online	NO ER+	NL	€ 1130	0.24	0.2	€ 4614 per QALY
Retèl [128]	2013	Adjuvant! Online	NO ER+	NL	€ –2401	0.62	–	Dominant
<i>Lymph node positive (or mixed)</i>								
Oestreicher [123]	2005	Best practice	N ≥ 0 stage ≤ II premenopausal	US	\$ –2882	–0.21	–	\$ 13,724 per QALY (in favor of BP)
OncotypeDX compared to no genomic testing								
<i>Lymph node negative</i>								
Bacchi [129]	2010	Usual care	NO ER+	Brazil	\$ –794	–	–	Cost saving
Cosler [130]	2009	Chemotherapy + Tamoxifen	NO ER+	US	\$ –2256	0	–	Dominant
Davidson [95]	2013	Usual care	NO ER+ HER2–	Canada	CAN\$ 2188	0.33	0.31	CAN\$ 6630 per QALY
Epstein [119]	2015	Usual care	NO ER+	US	\$ 1367	–	–	Cost increasing
Hannouf [131]	2012	Usual care	NO HR+	Canada	CAN\$ 2879	0.059	–	CAN\$ 48,493 per QALY
Holt [96]	2013	Usual care	NO-1 ER+	UK	£ 888	0.14	0.16	£ 6232 per QALY
Hornberger [132]	2005	Usual care	NO ER+	US	\$ –1160	0.162	–	Dominant
Hornberger [133]	2011	Best practice	NO ER+	US	\$ –2028	0.086	–0.0421	Dominant
Jahn [134]	2015	Adjuvant! Online	NO HR+ HER2–	Austria	€ 2750	0.46	0.59	€ 5978 per QALY
Katz [120]	2015	Usual care	NO HR+ HER2–	France	€ –602	0.17	0.18	Dominant
Klang [135]	2010	Usual care	NO ER+	Israel	\$ 1828	0.17	–	\$ 10,770 per QALY
Kondo [136]	2008	Best practice	NO HR+	Japan	\$ 2516	0.097	0.083	\$ 30,137 per QALY
Kondo [137]	2011	Best practice	NO ER+	Japan	\$ 2407	0.63	–	\$ 3848 per QALY
Lamond [138]	2012	Usual care	NO ER+	Canada	CAN\$ 2585	0.27	–	CAN\$ 9591 per QALY
OHTA [139]	2010	Adjuvant! Online	NO HR+ HER2–	Ontario	CAN\$ 4168	1.3	–	CAN\$ 3206 per QALY
Paulden [140]	2013	Adjuvant! Online	NO HR+ HER2–	Canada	CAN\$ 2460	0.429	0.53	CAN\$ 5734 per QALY
Reed [121]	2013	Adjuvant! Online	NO ER+	US	\$ 1741	0.16	0.19	\$ 10,788 per QALY
Smyth [141]	2015	Best practice	NO ER+	Ireland	€ –1361	–	–	Cost saving
Su [113]	2016	Usual care	NO HR+ HER2–	US	\$ 400	–	–	Cost increasing
Tsoi [142]	2010	Adjuvant! Online	NO HR+	Canada	CAN\$ 4102	0.065	0.064	CAN\$ 63,064 per QALY
Vataire [122]	2012	Usual care	NO ER+ HER2–	France	€ –1600	0.14	0.15	Dominant
Ward [143]	2013	Usual care	NO ER+ HER2–	UK	£ 2575	0.1	–	£ 29,502 per QALY
Yamauchi [144]	2014	Usual care	NO ER+	Japan	\$ 1536	0.241	–	\$ 6368 per QALY
<i>Lymph node positive (or mixed)</i>								
Bargalló-Rocha [145]	2015	Usual care	N3 HR+ HER2–	Mexico	\$ 129	–	0.068	\$ 1914 per LY
Blohmer [146]	2013	Usual care	N3 ER+ HER2–	Germany	€ –561	0.06	0.06	Dominant
Hall [147]	2012	Chemotherapy	N+ ER+	UK	£ 860	0.16	0.15	£ 5529 per QALY
Hannouf [148]	2014	Usual care	N+ HR+ post-menopausal	Canada	CAN\$ 36.2	0.08	–	CAN\$ 464 per QALY
Kip [149]	2015	Usual care	N1 ER+	NL	€ 1236	0.11	–	€ 11,236 per QALY
Kondo [137]	2011	Best practice	N+ ER+	Japan	\$ 3434	0.07	–	\$ 49,059 per QALY
Lamond [138]	2012	Usual care	N+ ER+	Canada	CAN\$ 864	0.06	–	CAN\$ 14,844 per QALY
Nerich [150]	2014	Usual care	N1 ER+ HER2–	France	€ –128	–	–	Cost saving
Vanderlaan [151]	2011	Best practice	N+ ER+ HER2–	US	\$ –384	0.127	–	Dominant
EndoPredict compared to no genomic testing								
<i>Lymph node positive (or mixed)</i>								
Blank [152]	2015	Best practice	N ≥ 0 ER+ HER2–	Germany	€ –3388	0.002	–0.037	Dominant
Head-to-head comparisons								
Mislick [153]	2014	Mammostrat vs OncotypeDX	NO ER+	US	\$ –2268	–0.005	–0.002	\$ 453,600 per QALY (in favor of Mammostrat)
Retèl [154]	2012	MammaPrint vs OncotypeDX	NO ER+	NL	€ –1475	0.08	–0.14	MammaPrint dominant
Seguí [155]	2014	MammaPrint vs OncotypeDX	NO ER+ HER2–	Spain	€ 1085	0.745	0.863	€ 1457 per QALY (in favor of MammaPrint)
Yang [156]	2012	MammaPrint vs OncotypeDX	NO ER+	US	\$ –6284	0.097	–	MammaPrint dominant

Fig. 2 shows the estimated impact of genomic testing on QALYs and costs, according to the 40 evaluations comparing genomic testing to a strategy without genomic testing. The horizontal axis shows the impact on QALYs: all studies but one [123] reported that genomic testing resulted in better patient outcome with a positive impact on QALYs. The vertical axis shows the impact on costs: genomic testing was cost saving in 14 (35%) evaluations and cost

increasing in 26 (65%) of the evaluations. On average, total costs increased by 449 euro per patient with an improvement on patient outcome of 0.16 life years and 0.20 QALYs. In general, there were no apparent differences between the estimated outcomes for the different genomic tests. Also, the range of costs was comparable in node-negative and node-positive patients, but the estimated QALY gain was larger in node-negative patients (on average, 0.24

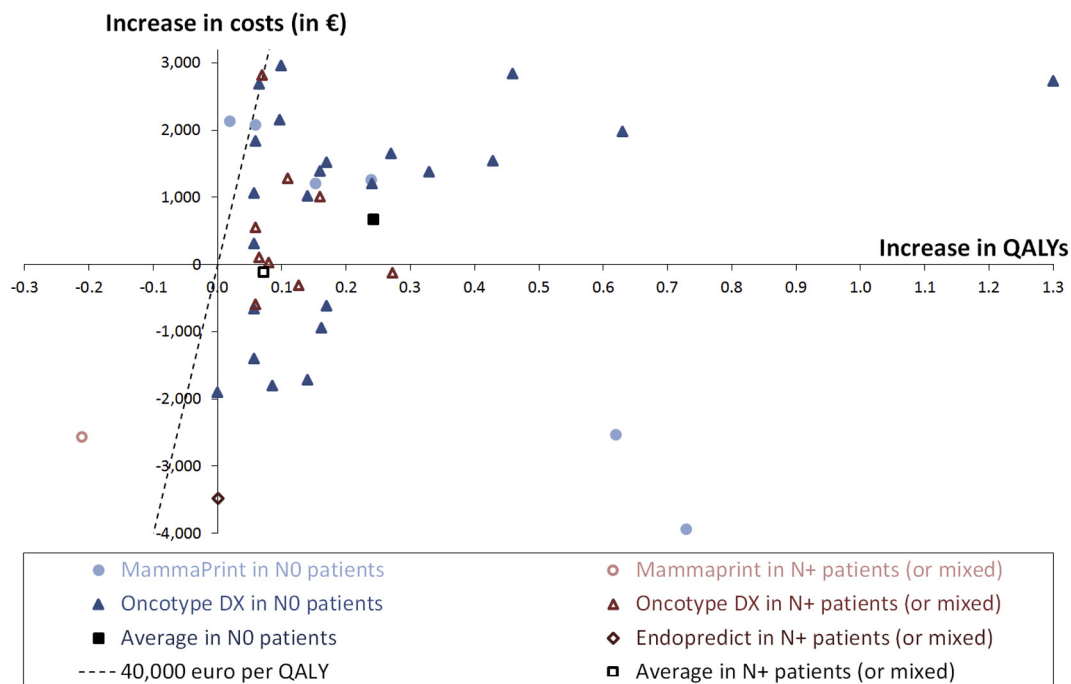


Fig. 2. Estimated impact on costs and quality-adjusted life years (QALYs) per economic evaluation, according to test and nodal status.

versus 0.07 QALYs). Considering the improvement in patient outcome, genomic testing was cost-effective in 36 (90%) of the evaluations, i.e. below the dashed 40,000 euro-per-QALY line.

Discussion

In this systematic review, we evaluated four commercially available prognostic genomic profiles on four selected crucial aspects. On all aspects, the tests are well-studied, with multiple well-designed and well-performed studies available. It is apparent that on the level of quantity, MammaPrint and especially OncotypeDX are more extensively studied compared to the more recently developed Endopredict and Prosigna/PAM50 assay. At this time of development, both OncotypeDX and MammaPrint are suitable assays which can be helpful in the clinical setting. However, this review also identified some caveats which will need to be addressed before genomic profiling can be optimally applied.

Assay development and methodology

The first topic for improvement is the identification of a subgroup that benefits most from genomic profiling. This has already been investigated for OncotypeDX, and to a lesser extent for MammaPrint. For Prosigna and Endopredict we did not identify studies that studied for which clinicopathological subtypes genomic profiling is valuable. In general, the studies show that patients with grade 3, PR- and a high Ki-67 have no benefit from testing, since they are almost always high-risk. In contrast, patients with grade 1, ER+PR+ and Ki-67 <10% have no benefit from testing either, since (almost) all of them had a low-risk result. As suggested by the flowchart build by Allison et al., all other patients would have an indication for genomic profiling [38]. However, most of these studies were performed in a node-negative cohort. MINDACT has shown that despite node-positive disease, it could be considered to withhold chemotherapy at a low genomic risk score. Therefore, it is crucial that this test-result predicting model is validated and adjusted in large trial cohorts like MINDACT and the WSG Plan-B trial.

Clinical validation

One of the most important (theoretical) benefits of a genomic profiling test is the selection of patients in which the treatment with adjuvant chemotherapy will have a significant benefit. Currently, this task of genomic profiles is mainly performed by their prognostic capacities; i.e. the ability to identify patients with a poor prognosis for recurrence or survival. However, the results of the studies in this review, especially that of MINDACT, show that this does not automatically translate into a benefit of chemotherapy for these higher-risk patients. So far, no genomic test has shown its predictive capacities in a prospective trial design. The only evidence for a predictive value was obtained in two prospective studies conducted on archived tissue (prospective-retrospective design) in which the OncotypeDX retrospectively identified patients that benefit more from chemotherapy to which they were randomly allocated [62,65].

Clinical utility

Currently, the clinical consensus on adjuvant chemotherapy is that we are most likely over-treating our patients, since we are not capable of identifying patients that will or will not benefit from chemotherapy using the current clinicopathological parameters [157,158]. It is no surprise that the studies evaluating the clinical utility of genomic profiling especially show a reduction in chemotherapy use. However, absolute numbers should be interpreted carefully, since some tests are less frequently studied than others, which increases the risk of bias and skewed data. Interestingly, in retrospective population-based cohorts, implementation of genomic testing did not lead to a reduction in chemotherapy use [113–115]. This is in accordance with Petkov et al., who retrospectively matched OncotypeDX use with SEER registry data for over 40,000 patients [159]. Although the risk categories were indeed prognostic for five-year breast-cancer-specific mortality in this real-life population, patients with node negative, HR+, HER2-breast cancer which underwent testing (n=40,134, 22.7% chemotherapy) had no lower chemotherapy use compared to

patients that were not tested ($n = 144,056$, 22.2% chemotherapy). Therefore, conclusions about genomic profiling leading to decrease in chemotherapy cannot be drawn from these analyses.

Economic value

Our review of economic evaluations identified 44 original publications, where earlier reviews included at most 11 or 18 published evaluations [160,161]. Except for the oldest evaluation [123], all studies reported improved patient outcome in terms of QALYs. Despite estimated savings on chemotherapy, recurrence and productivity, a small majority (65%) of the evaluations estimated that genomic testing resulted in an increase in total costs. Nevertheless, most evaluations (90%) estimated that genomic testing is cost-effective, with costs that are acceptable in relation to patient outcome. These economic results should be considered with caution. Firstly, the separate evaluations should not be interpreted as independent primary studies, because the models obtain their data from overlapping sources: mostly the diagnostic data are taken from the landmark trials and then applied to the care patterns of a particular country. Secondly, the economic studies generally evaluate the use of genomic testing in large groups of women, instead of trying to combine genomic profiling with other prognostic factors to identify those individual women for whom genomic testing does not have sufficient added value or could even be harmful. And thirdly, compared to trials, economic evaluations are more likely to suffer from publication bias.

Future perspectives

In the near future, trial results from RxPonder, TAILORx and WSG plan-B will become available, contributing to understanding the role of OncotypeDX in daily practice in both node-positive and node-negative disease. Furthermore, subgroup analyses and long-term follow-up of MINDACT will follow later and help define the place for MammaPrint in the diagnostic process, and the long-term safety of withholding chemotherapy in high-risk patients, based on a low-risk test result. The OPTIMA trial, randomizing high-risk ER+HER2- patients between standard chemotherapy, or treatment directed by Prosigna test-results will be the first trial to show level A evidence for the Prosigna/PAM50 test.

Another interesting development is the use of gene expression assays for the indication of endocrine therapy. Very recently, a retrospective analysis from Sweden identified an ultra-low category within the low-risk category of MammaPrint (15% of all patients, 26% of low-risk patients) [162]. Patients with this ultra-low risk score ($n = 98$) had a breast cancer specific survival of 94% at 20 years without any adjuvant therapy, and 97% at 20 years with just 2 years of tamoxifen, whereas 5+ years of therapy is the current standard for these patients [163]. Upon validation, these findings could lead to the implementation of gene expression assays in the indication for adjuvant endocrine therapy.

Conclusions

In summary, in this systematic review we have evaluated the four most frequently used assays in Europe on four relevant aspects. Regarding the amount of evidence, there is a clear separation between the more established MammaPrint and OncotypeDX on one hand, and the newer Prosigna and Endopredict on the other hand. Comparing MammaPrint and OncotypeDX, both assays have shown to be a useful prognostic tests which could lead to a reduction in chemotherapy use, with in general a favourable cost-benefit ratio. Both the MammaPrint and OncotypeDX have shown in prospective trials that a patient with a low-risk result can safely forego chemotherapy, despite clinical risk factors. In contrast, the

benefit of chemotherapy with a high-risk test result has so far only been shown for OncotypeDX, albeit in retrospective analyses of archived tissue of prospective trials. Therefore, there is still a need for further prospective studies on all evaluated assays.

Conflicts of interest

None.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ctrv.2017.10.012>.

References

- [1] (EBCTCG) EBCTCG. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*. 2005;365:1687–717.
- [2] Early Breast Cancer Trialists' Collaborative G. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100000 women in 123 randomised trials. *Lancet*. 2012;379:432–44.
- [3] Reis-Filho JS, Pusztai L. Gene expression profiling in breast cancer: classification, prognostication, and prediction. *The Lancet*. 378:1812–23.
- [4] Ballman KV. Biomarker: predictive or prognostic? *J Clin Oncol* 2015;33:3968–71.
- [5] van 't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;415:530–6.
- [6] van de Vijver MJ, He YD, van't Veer LJ, Dai H, Hart AA, Voskuil DW, 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, Kim C, Baker J, Cronin M, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med* 2004;351:2817–26.
- [8] Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Prospective validation of a 21-gene expression assay in breast cancer. *N Engl J Med* 2015;373:2005–14.
- [9] Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol* 2009;27:1160–7.
- [10] Nielsen T, Wallden B, Schaper C, Ferree S, Liu S, Gao D, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer* 2014;14:177.
- [11] Filipits M, Rudas M, Jakesz R, Dubsy P, Fitzal F, Singer CF, et al. 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;17:6012–20.
- [12] Glas AM, Floore A, Delahaye LJM, Witteveen AT, Pover RCF, Bakx N, et al. Converting a breast cancer microarray signature into a high-throughput diagnostic test. *Bmc Genomics* 2006;7:278.
- [13] Ach RA, Floore A, Curry B, Lazar V, Glas AM, Pover R, et al. Robust interlaboratory reproducibility of a gene expression signature measurement consistent with the needs of a new generation of diagnostic tools. *BMC Genomics* 2007;8:148.
- [14] Mittempergher L, de Ronde JJ, Nieuwland M, Kerkhoven RM, Simon I, Rutgers EJ, et al. Gene expression profiles from formalin fixed paraffin embedded breast cancer tissue are largely comparable to fresh frozen matched tissue. *PLoS ONE* 2011;6:e17163.
- [15] Sapino A, Roepman P, Linn SC, Snel MH, Delahaye LJ, van den Akker J, et al. MammaPrint molecular diagnostics on formalin-fixed, paraffin-embedded tissue. *J Mol Diagn* 2014;16:190–7.
- [16] Beumer I, Witteveen A, Delahaye L, Wehkamp D, Snel M, Dreezen C, et al. Equivalence of MammaPrint array types in clinical trials and diagnostics. *Breast Cancer Res Treat* 2016;156:279–87.

- [17] Gyanchandani R, Lin Y, Lin HM, Cooper K, Normolle DP, Brufsky A, et al. Intratumor heterogeneity affects gene expression profile test prognostic risk stratification in early breast cancer. *Clin Cancer Res* 2016;22:5362–9.
- [18] Drury S, Salter J, Baehner FL, Shak S, Dowsett M. Feasibility of using tissue microarray cores of paraffin-embedded breast cancer tissue for measurement of gene expression: a proof-of-concept study. *J Clin Pathol* 2010;63:513–7.
- [19] Müller BM, Brase JC, Haufe F, Weber KE, Budzies J, Petry C, et al. Comparison of the RNA-based EndoPredict multigene test between core biopsies and corresponding surgical breast cancer sections. *J Clin Pathol* 2012;65:660–2.
- [20] Denkert C, Kronenwett R, Schlake W, Bohmann K, Penzel R, Weber KE, et al. Decentral gene expression analysis for ER+/Her2- breast cancer: results of a proficiency testing program for the EndoPredict assay. *Virchows Arch* 2012;460:251–9.
- [21] Kronenwett R, Bohmann K, Prinzler J, Sinn BV, Haufe F, Roth C, et al. Decentral gene expression analysis: analytical validation of the Endopredict genomic multianalyte breast cancer prognosis test. *BMC Cancer* 2012;12:456.
- [22] Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, et al. 70-Genes signature as an aid to treatment decisions in early-stage breast cancer. *N Engl J Med*. 2016;375:717–29.
- [23] Elloumi F, Hu Z, Li Y, Parker JS, Gulley ML, Amos KD, et al. Systematic bias in genomic classification due to contaminating non-neoplastic tissue in breast tumor samples. *BMC Med Genomics*. 2011;4:54-.
- [24] Stein RC, Dunn JA, Bartlett JM, Campbell AF, Marshall A, Hall P, et al. OPTIMA prelim: a randomised feasibility study of personalised care in the treatment of women with early breast cancer. *Health Technol Assess (Winchester, England)*. 2016;20:xxiii–xxix, 1–201.
- [25] Bartlett JMS, Stein RC, Bayani J, Marshall A, Dunn JA, Campbell AF, et al. Comparison of multiparameter tests in the UK OPTIMA-Prelim trial. *Can Res* 2015;75.
- [26] Alvarado MD, Prasad C, Rothney M, Cherbavaz DB, Sing AP, Baehner FL, et al. A prospective comparison of the 21-gene recurrence score and the PAM50-based prognostic index in estrogen receptor-positive early-stage breast cancer. *Adv Ther* 2015;32:1237–47.
- [27] Varga Z, Sinn P, Fritzsche F, von Hochstetter A, Noske A, Schraml P, et al. Comparison of EndoPredict and Oncotype DX test results in hormone receptor positive invasive breast cancer. *PLoS ONE* 2013;8:e58483.
- [28] Gevensleben H, Gohring UJ, Buttner R, Heukamp LC, Kunz G, Dimpfl T, et al. Comparison of MammaPrint and TargetPrint results with clinical parameters in German patients with early stage breast cancer. *Int J Mol Med* 2010;26:837–43.
- [29] Chaudhary LN, Jawa Z, Szabo A, Visotcky A, Chitambar CR. Relevance of progesterone receptor immunohistochemical staining to Oncotype DX recurrence score. *Hematol Oncol Stem Cell Ther* 2016;9:48–54.
- [30] Dialani V, Gaur S, Mehta TS, Venkataraman S, Fein-Zachary V, Phillips J, et al. Prediction of low versus high recurrence scores in estrogen receptor-positive, lymph node-negative invasive breast cancer on the basis of radiologic-pathologic features: comparison with oncotype DX test recurrence scores. *Radiology* 2016;280:370–8.
- [31] Ingoldsby H, Webber M, Wall D, Scarrott C, Newell J, Callagy G. Prediction of Oncotype DX and TAILORx risk categories using histopathological and immunohistochemical markers by classification and regression tree (CART) analysis. *Breast* 2013;22:879–86.
- [32] Sahebjam S, Aloyz R, Pilavdzic D, Brisson ML, Ferrario C, Bouganin N, et al. Ki 67 is a major, but not the sole determinant of Oncotype Dx recurrence score. *Br J Cancer* 2011;105:1342–5.
- [33] Auerbach J, Kim M, Fineberg S. Can features evaluated in the routine pathologic assessment of lymph node-negative estrogen receptor-positive Stage I or II invasive breast cancer be used to predict the oncotype DX recurrence score? *Arch Pathol Lab Med* 2010;134:1697–701.
- [34] Flanagan MB, Dabbs DJ, Brufsky AM, Beriwal S, Bhargava R. Histopathologic variables predict Oncotype DX recurrence score. *Mod Pathol* 2008;21:1255–61.
- [35] Wolf I, Ben-Baruch N, Shapira-Frommer R, Rizel S, Goldberg H, Yaal-Hahoshen N, et al. Association between standard clinical and pathologic characteristics and the 21-gene recurrence score in breast cancer patients: a population-based study. 2008;112:731–6.
- [36] Gluz O, Nitz UA, Christgen M, Kates RE, Shak S, Clemens M, et al. West German study group phase III PlanB 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;34:2341–9.
- [37] Bradshaw SH, Pidutti D, Gravel DH, Song X, Marginean EC, Robertson SJ. Predicting OncoDx recurrence scores with immunohistochemical markers. *Appl Immunohistochem Mol Morphol*: A1MM 2013;21:490–6.
- [38] Allison KH, Kandalaft PL, Sitlani CM, Dintzis SM, Gown AM. Routine pathologic parameters can predict Oncotype DX recurrence scores in subsets of ER positive patients: who does not always need testing? 2012;131:413–24.
- [39] Williams DJ, Cohen C, Darrow M, Page AJ, Chastain B, Adams AL. Proliferation (Ki-67 and phosphohistone H3) and oncotype DX recurrence score in estrogen receptor-positive breast cancer. *Appl Immunohistochem Mol Morphol*: A1MM 2011;19:431–6.
- [40] Wittner BS, Sgroi DC, Ryan PD, Bruijnsmas TJ, Glas AM, Male A, et al. Analysis of the MammaPrint breast cancer assay in a predominantly postmenopausal cohort. *Clin Cancer Res* 2008;14:2988–93.
- [41] Mook S, Schmidt MK, Weigelt B, Kreike B, Eekhout I, van de Vijver MJ, et al. The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. *Ann Oncol* 2010;21:717–22.
- [42] Na KY, Kim KS, Lee JE, Kim HJ, Yang JH, Ahn SH, et al. The 70-gene prognostic signature for Korean breast cancer patients. *J Breast Cancer* 2011;14:33–8.
- [43] Drukker CA, Bueno-de-Mesquita JM, Retel VP, van Harten WH, van Tinteren H, Wesseling J, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. *Int J Cancer* 2013;133:929–36.
- [44] Bueno-de-Mesquita JM, van Harten WH, Retel VP, van't Veer LJ, van Dam FS, Karsenberg K, 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;8:1079–87.
- [45] Bueno-de-Mesquita JM, Linn SC, Keijzer R, Wesseling J, Nuyten DS, van Krimpen C, et al. Validation of 70-gene prognosis signature in node-negative breast cancer. *Breast Cancer Res Treat* 2009;117:483–95.
- [46] Knauer M, Mook S, Rutgers EJ, Bender RA, Hauptmann M, van de Vijver MJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. *Breast Cancer Res Treat* 2010;120:655–61.
- [47] Saghatchian M, Mook S, Pruneri G, Viale G, Glas AM, Guerin S, et al. Additional prognostic value of the 70-gene signature (MammaPrint (R)) among breast cancer patients with 4–9 positive lymph nodes. *Breast* 2013;22:682–90.
- [48] Mook S, Schmidt MK, Viale G, Pruneri G, Eekhout I, Floore A, 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.
- [49] Mook S, Knauer M, Bueno-de-Mesquita JM, Retel VP, Wesseling J, Linn SC, et al. Metastatic potential of T1 breast cancer can be predicted by the 70-gene MammaPrint Signature. *Ann Surg Oncol* 2010;17:1406–13.
- [50] Knauer M, Cardoso F, Wesseling J, Bedard PL, Linn SC, Rutgers EJ, et al. Identification of a low-risk subgroup of HER-2-positive breast cancer by the 70-gene prognosis signature. *Br J Cancer* 2010;103:1788–93.
- [51] Drukker CA, van Tinteren H, Schmidt MK, Rutgers EJ, Bernards R, van de Vijver MJ, et al. Long-term impact of the 70-gene signature on breast cancer outcome. *Breast Cancer Res Treat* 2014;143:587–92.
- [52] Drukker CA, Elias SG, Nijenhuis MV, Wesseling J, Bartelink H, Elkhuizen P, et al. Gene expression profiling to predict the risk of locoregional recurrence in breast cancer: a pooled analysis. *Breast Cancer Res Treat* 2014;148:599–613.
- [53] Buyse M, Loi S, van't Veer L, Viale G, Delorenzi M, Glas AM, et al. Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. *J Natl Cancer Inst* 2006;98:1183–92.
- [54] Kunz G. Use of a genomic test (MammaPrint (TM)) in daily clinical practice to assist in risk stratification of young breast cancer patients. *Arch Gynecol Obstet* 2011;283:597–602.
- [55] Kok M, Koornstra RH, Mook S, Hauptmann M, Fles R, Jansen MP, et al. Additional value of the 70-gene signature and levels of ER and PR for the prediction of outcome in tamoxifen-treated ER-positive breast cancer. *Breast* 2012;21:769–78.
- [56] Ahn SG, Lee HM, Lee HW, Lee SA, Lee SR, Leem SH, et al. Prognostic discrimination using a 70-gene signature among patients with estrogen receptor-positive breast cancer and an intermediate 21-gene recurrence score. *Int J Mol Sci* 2013;14:23685–99.
- [57] Ishitobi M, Goranova TE, Komoike Y, Motomura K, Koyama H, Glas AM, et al. Clinical utility of the 70-gene MammaPrint profile in a Japanese population. *Jpn J Clin Oncol* 2010;40:508–12.
- [58] Toi M, Iwata H, Yamanaka T, Masuda N, Ohno S, Nakamura S, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. *Cancer* 2010;116:3112–8.
- [59] Naoi Y, Kishi K, Tsunashima R, Shimazu K, Shimomura A, Maruyama N, et al. Comparison of efficacy of 95-gene and 21-gene classifier (Oncotype DX) for prediction of recurrence in ER-positive and node-negative breast cancer patients. *Breast Cancer Res Treat* 2013;140:299–306.
- [60] Mamounas EP, Tang G, Fisher B, Paik S, Shak S, Costantino JP, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. *J Clin Oncol* 2010;28:1677–83.
- [61] Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schinabel CA, Schroeder B, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol* 2013;14:1067–76.
- [62] Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol* 2006;24:3726–34.
- [63] Tang G, Cuzick J, Costantino JP, Dowsett M, Forbes JF, Cragger M, et al. Risk of recurrence and chemotherapy benefit for patients with node-negative, estrogen receptor-positive breast cancer: recurrence score alone and integrated with pathologic and clinical factors. *J Clin Oncol* 2011;29:4365–72.
- [64] Yorozuya K, Takeuchi T, Yoshida M, Mouri Y, Kousaka J, Fujii K, et al. Evaluation of Oncotype DX Recurrence Score as a prognostic factor in Japanese women with estrogen receptor-positive, node-negative primary Stage I or IIA breast cancer. *J Cancer Res Clin* 2010;136:939–44.
- [65] Albain KS, Barlow WE, Shak S, Hortobagyi GN, Livingston RB, Yeh IT, 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.
- [66] Dowsett M, Cuzick J, Wale C, Forbes J, Mallon EA, Salter J, et al. 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;28:1829–34.
- [67] Cuzick J, Dowsett M, Pineda S, Wale C, Salter J, Quinn E, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the genomic health recurrence score in early breast cancer. *J Clin Oncol* 2011;29:4273–8.
- [68] Goldstein LJ, Gray R, Badve S, Childs BH, Yoshizawa C, Rowley S, et al. Prognostic utility of the 21-gene assay in hormone receptor-positive operable breast cancer compared with classical clinicopathologic features. *J Clin Oncol* 2008;26:4063–71.
- [69] Le Du F, Gonzalez-Angulo AM, Park M, Liu DD, Hortobagyi GN, Ueno NT. Effect of 21-gene RT-PCR assay on adjuvant therapy and outcomes in patients with stage I breast cancer. *Clin Breast Cancer* 2015;15:458–66.
- [70] Tang G, Shak S, Paik S, Anderson SJ, Costantino JP, Geyer Jr CE, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat* 2011;127:133–42.
- [71] Freitas MR, Simon SD. Comparison between Oncotype DX test and standard prognostic criteria in estrogen receptor positive early-stage breast cancer. *Einstein (Sao Paulo)* 2011;9:354–8.
- [72] Aktas B, Bankfalvi A, Heubner M, Kimmig R, Kasimir-Bauer S. Evaluation and correlation of risk recurrence in early breast cancer assessed by Oncotype DX (R), clinicopathological markers and tumor cell dissemination in the blood and bone marrow. *Mol Clin Oncol* 2013;1:1049–54.
- [73] Acs G, Kiluk J, Loftus L, Laronga C. Comparison of Oncotype DX and Mammostrat risk estimations and correlations with histologic tumor features in low-grade, estrogen receptor-positive invasive breast carcinomas. *Mod Pathol* 2013;26:1451–60.
- [74] Kok M, Linn SC, Van Laar RK, Jansen MP, van den Berg TM, Delahaye LJ, et al. Comparison of gene expression profiles predicting progression in breast cancer patients treated with tamoxifen. *Breast Cancer Res Treat* 2009;113:275–83.
- [75] Martin M, Prat A, Rodriguez-Lescure A, Caballero R, Ebbert MT, Munarriz B, et al. PAM50 proliferation score as a predictor of weekly paclitaxel benefit in breast cancer. *Breast Cancer Res Treat* 2013;138:457–66.
- [76] Liu S, Chapman JA, Burnell MJ, Levine MN, Pritchard KI, Whelan TJ, et al. Prognostic and predictive investigation of PAM50 intrinsic subtypes in the NCIC CTG MA.21 phase III chemotherapy trial. *Breast Cancer Res Treat* 2015;149:439–48.
- [77] Sestak I, Cuzick J, Dowsett M, Lopez-Knowles E, Filipits M, Dubsy P, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. *J Clin Oncol* 2015;33:916–22.
- [78] Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, et al. 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: Off J Eur Soc Med Oncol* 2014;25:339–45.
- [79] Filipits M, Nielsen TO, Rudas M, Greil R, Stoger H, Jakesz R, et al. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. *Clin Cancer Res* 2014;20:1298–305.
- [80] Fitzal F, Filipits M, Rudas M, Greil R, Dietze O, Samonigg H, et al. The genomic expression test EndoPredict is a prognostic tool for identifying risk of local recurrence in postmenopausal endocrine receptor-positive, her2neu-negative breast cancer patients randomised within the prospective ABCSG 8 trial. *Br J Cancer* 2015;112:1405–10.
- [81] Dubsy P, Filipits M, Jakesz R, Rudas M, Singer CF, Greil R, et al. EndoPredict improves the prognostic classification derived from common clinical guidelines in ER-positive, HER2-negative early breast cancer. *Ann Oncol: Off J Eur Soc Med Oncol* 2013;24:640–7.
- [82] Dubsy P, Brase JC, Jakesz R, Rudas M, Singer CF, Greil R, et al. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2– breast cancer patients. *Br J Cancer* 2013;109:2959–64.
- [83] Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst* 2009;101:1446–52.
- [84] Gonzalez-Angulo AM, Barlow WE, Gralow J, Meric BF, Hayes DF, Moynour C, et al. SWOG S1007: a phase III, randomized clinical trial of standard adjuvant endocrine therapy with or without chemotherapy in patients with one to three positive nodes, hormone receptor (HR)-positive, and HER2-negative breast cancer with recurrence score (RS) of 25 or less. *J Clin Oncol* 2011;29.
- [85] Drukker CA, van den Hout HC, Sonke GS, Brain E, Bonnefoi H, Cardoso F, et al. Risk estimations and treatment decisions in early stage breast cancer: agreement among oncologists and the impact of the 70-gene signature. *Eur J Cancer* 2014;50:1045–54.
- [86] Pohl H, Kotze MJ, Grant KA, van der Merwe L, Pienaar FM, Appfelstaedt JP, et al. Impact of MammaPrint on clinical decision-making in South African patients with early-stage breast cancer. *Breast J* 2016;22:442–6.
- [87] Exner R, Bago-Horvath Z, Bartsch R, Mittlboeck M, Retel VP, Fitzal F, et al. The multigene signature MammaPrint impacts on multidisciplinary team decisions in ER+, HER2– early breast cancer. *Br J Cancer* 2014;111:837–42.
- [88] Cusumano PG, Generali D, Ciruelos E, Manso L, Ghnam I, Lifrange E, et al. European inter-institutional impact study of MammaPrint. *Breast* 2014;23:423–8.
- [89] Ozmen V, Atasoy A, Gokmen E, Ozdogan M, Guler N, Uras C, et al. Impact of oncotype DX recurrence score on treatment decisions: results of a prospective multicenter study in Turkey. *Cureus* 2016;8:e522.
- [90] Levine MN, Julian JA, Bedard PL, Eisen A, Trudeau ME, Higgins B, et al. Prospective evaluation of the 21-gene recurrence score assay for breast cancer decision-making in Ontario. *J Clin Oncol* 2016;34:1065–71.
- [91] Leung RC, Yau TC, Chan MC, Chan SW, Chan TW, Tsang YY, et al. The impact of the oncotype DX breast cancer assay on treatment decisions for women with estrogen receptor-positive, node-negative breast carcinoma in Hong Kong. *Clin Breast Cancer* 2016;16:372–8.
- [92] Gligorov J, Pivot XB, Jacot W, Naman HL, Spaeth D, Misset JL, et al. Prospective clinical utility study of the use of the 21-gene assay in adjuvant clinical decision making in women with estrogen receptor-positive early invasive breast cancer: results from the SWITCH study. *Oncologist* 2015;20:873–9.
- [93] Lee MH, Han W, Lee JE, Kim KS, Park H, Kim J, et al. The clinical impact of 21-gene recurrence score on treatment decisions for patients with hormone receptor-positive early breast cancer in Korea. *Cancer Res Treat* 2015;47:208–14.
- [94] Jaafar H, Bashir MA, Taher A, Qawasmeh K, Jaloudi M. Impact of Oncotype DX testing on adjuvant treatment decisions in patients with early breast cancer: a single-center study in the United Arab Emirates. *Asia Pac J Clin Oncol* 2014;10:354–60.
- [95] Davidson JA, Cromwell I, Ellard SL, Lohrisch C, Gelmon KA, Shenkier T, et al. A prospective clinical utility and pharmaco-economic study of the impact of the 21-gene Recurrence Score(R) assay in oestrogen receptor positive node negative breast cancer. *Eur J Cancer* 2013;49:2469–75.
- [96] Holt S, Bertelli G, Humphreys I, Valentine W, Durrani S, Pudney D, et al. A decision impact, decision conflict and economic assessment of routine Oncotype DX testing of 146 women with node-negative or pN1mi, ER-positive breast cancer in the U.K. *Br J Cancer* 2013;108:2250–8.
- [97] Birschack JR, Schwartz GF, Palazzo JP, Toll AD, Brill KL, Jaslow RJ, et al. Impact of Oncotype DX on treatment decisions in ER-positive, node-negative breast cancer with histologic correlation. *Breast J* 2013;19:269–75.
- [98] Ademuyiwa FO, Miller A, O'Connor T, Edge SB, Thorat MA, Sledge GW, et al. The effects of oncotype DX recurrence scores on chemotherapy utilization in a multi-institutional breast cancer cohort. *Breast Cancer Res Treat* 2011;126:797–802.
- [99] Albanell J, Gonzalez A, Ruiz-borrego M, Alba E, Garcia-saenz JA, Corominas JM, et al. Prospective transGEICAM study of the impact of the 21-gene recurrence score assay and traditional clinicopathological factors on adjuvant clinical decision making in women with estrogen receptor-positive (ER+) node-negative breast cancer. *Ann Oncol* 2012;23:625–31.
- [100] Lo SS, Mumby PB, Norton J, Rychlik K, Smerage J, Kash J, et al. Prospective multicenter study of the impact of the 21-gene recurrence score assay on medical oncologist and patient adjuvant breast cancer treatment selection. *J Clin Oncol* 2010;28:1671–6.
- [101] Henry LR, Stojadinovic A, Swain SM, Prindiville S, Cordes R, Soballe PW. The influence of a gene expression profile on breast cancer decisions. *J Surg Oncol* 2009;99:319–23.
- [102] Oratz R, Paul D, Cohn AL, Sedlacek SM. Impact of a commercial reference laboratory test recurrence score on decision making in early-stage breast cancer. *J Oncol Pract* 2007;3:182–6.
- [103] Kuchel A, Robinson T, Comins C, Shere M, Varughese M, Sparrow G, et al. The impact of the 21-gene assay on adjuvant treatment decisions in oestrogen receptor-positive early breast cancer: a prospective study. *Br J Cancer* 2016;114:731–6.
- [104] Bargallo JE, Lara F, Shaw-Dulin R, Perez-Sanchez V, Villarreal-Garza C, Maldonado-Martinez H, et al. A study of the impact of the 21-gene breast cancer assay on the use of adjuvant chemotherapy in women with breast cancer in a Mexican public hospital. *J Surg Oncol* 2015;111:203–7.
- [105] Yamauchi H, Nakagawa C, Takei H, Chao C, Yoshizawa C, Yagata H, et al. Prospective study of the effect of the 21-gene assay on adjuvant clinical decision-making in Japanese women with estrogen receptor-positive, node-negative, and node-positive breast cancer. *Clin Breast Cancer* 2014;14:191–7.
- [106] Fried G, Moskovitz M. Treatment decisions in estrogen receptor-positive early breast cancer patients with intermediate oncotype DX recurrence score results. *Springerplus* 2014;3:71.
- [107] Cheung PS, Tong AC, Leung RC, Kwan WH, Yau TC. Initial experience with the Oncotype DX assay in decision-making for adjuvant therapy of early oestrogen receptor-positive breast cancer in Hong Kong. *Hong Kong Med J* 2014;20:401–6.
- [108] Eiermann W, Rezai M, Kummel S, Kuhn T, Warm M, Friedrichs K, et al. The 21-gene recurrence score assay impacts adjuvant therapy recommendations for ER-positive, node-negative and node-positive early breast cancer resulting in a risk-adapted change in chemotherapy use. *Ann Oncol* 2013;24:618–24.

- [109] de Boer RH, Baker C, Speakman D, Chao CY, Yoshizawa C, Mann GB. The impact of a genomic assay (Oncotype DX) on adjuvant treatment recommendations in early breast cancer. *Med J Aust* 2013;199:205–8.
- [110] Geffen DB, Abu-Ghanem S, Sion-Vardy N, Braunstein R, Tokar M, Ariad S, et al. The impact of the 21-gene recurrence score assay on decision making about adjuvant chemotherapy in early-stage estrogen-receptor-positive breast cancer in an oncology practice with a unified treatment policy. *Ann Oncol* 2011;22:2381–6.
- [111] Martin M, Gonzalez-Rivera M, Morales S, de la Haba-Rodriguez J, Gonzalez-Cortijo L, Manso L, et al. Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor positive, human epidermal growth factor receptor negative, node negative early-stage breast cancer. *Curr Med Res Opin* 2015;31:1129–37.
- [112] Muller BM, Keil E, Lehmann A, Winzer KJ, Richter-Ehrenstein C, Prinzler J, et al. The EndoPredict gene-expression assay in clinical practice – performance and impact on clinical decisions. *PLoS ONE* 2013;8:e68252.
- [113] Su KW, Hall J, Soulos PR, Abu-Khalaf MM, Evans SB, Mougalian SS, et al. Association of 21-gene recurrence score assay and adjuvant chemotherapy use in the medicare population, 2008–2011. *J Geriatr Oncol* 2016;7:15–23.
- [114] Potosky AL, O'Neill SC, Isaacs C, Tsai HT, Chao C, Liu CF, et al. Population-based study of the effect of gene expression profiling on adjuvant chemotherapy use in breast cancer patients under the age of 65 years. *Cancer* 2015;121:4062–70.
- [115] Hassett MJ, Silver SM, Hughes ME, Blayney DW, Edge SB, Herman JG, et al. Adoption of gene expression profile testing and association with use of chemotherapy among women with breast cancer. *J Clin Oncol* 2012;30:2218–26.
- [116] Stemmer SM, Klang SH, Ben-Baruch N, Geffen DB, Steiner M, Soussan-Gutman L, et al. 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.
- [117] Ray GT, Mandelblatt J, Habel LA, Ramsey S, Kushi LH, Li Y, et al. Breast cancer multigene testing trends and impact on chemotherapy use. *Am J Manage Care* 2016;22:e153–60.
- [118] Kuijter A, van Bommel AC, Drukker CA, van der Heiden-van der Loo M, Smorenburg CH, Westenend PJ, et al. Using a gene expression signature when controversy exists regarding the indication for adjuvant systemic treatment reduces the proportion of patients receiving adjuvant chemotherapy: a nationwide study. *Genet Med* 2016;18:720–6.
- [119] Epstein AJ, Wong YN, Mitra N, Vachani A, Hin S, Yang L, et al. Adjuvant chemotherapy use and health care costs after introduction of genomic testing in breast cancer. *J Clin Oncol*. 2015;33:4259–+.
- [120] Katz G, Romano O, Foa C, Vataire AL, Chantelard JV, Herve R, et al. Economic impact of gene expression profiling in patients with early-stage breast cancer in France. *PLoS ONE* 2015;10:e0128880.
- [121] Reed SD, Dinan MA, Schulman KA, Lyman GH. Cost-effectiveness of the 21-gene recurrence score assay in the context of multifactorial decision making to guide chemotherapy for early-stage breast cancer. *Genet Med* 2013;15:203–11.
- [122] Vataire AL, Laas E, Aballea S, Gligorov J, Rouzier R, Chereau E. Cost-effectiveness of a chemotherapy predictive test. *Bull Cancer* 2012;99:907–14.
- [123] Oestreicher N, Ramsey SD, Linden HM, McCune JS, van't Veer LJ, Burke W, et al. Gene expression profiling and breast cancer care: what are the potential benefits and policy implications? *Genet Med* 2005;7:380–9.
- [124] Bonastre J, Marguet S, Lueza B, Michiels S, Delaloue S, Saghatian M. Cost effectiveness of molecular profiling for adjuvant decision making in patients with node-negative breast cancer. *J Clin Oncol* 2014;32:3513–9.
- [125] Chen E, Tong KB, Malin JL. Cost-effectiveness of 70-gene MammaPrint signature in node-negative breast cancer. *Am J Manage Care* 2010;16:e333–42.
- [126] Kondo M, Hoshi SL, Ishiguro H, Toi M. Economic evaluation of the 70-gene prognosis-signature (MammaPrint(R)) in hormone receptor-positive, lymph node-negative, human epidermal growth factor receptor type 2-negative early stage breast cancer in Japan. *Breast Cancer Res Treat* 2012;133:759–68.
- [127] Retel 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;46:1382–91.
- [128] Retel VP, Joore MA, Drukker CA, Bueno-de-Mesquita JM, Knauer M, van Tinteren H, et al. Prospective cost-effectiveness analysis of genomic profiling in breast cancer. *Eur J Cancer* 2013;49:3773–9.
- [129] Bacchi CE, Prisco F, Carvalho FM, Ojopi EB, Saad ED. Potential economic impact of the 21-gene expression assay on the treatment of breast cancer in Brazil. *Rev Assoc Med Bras* 1992;2010(56):186–91.
- [130] Cosler LE, Lyman GH. Economic analysis of gene expression profile data to guide adjuvant treatment in women with early-stage breast cancer. *Cancer Invest* 2009;27:953–9.
- [131] Hannouf MB, Xie B, Brackstone M, Zaric GS. Cost-effectiveness of a 21-gene recurrence score assay versus Canadian clinical practice in women with early-stage estrogen- or progesterone-receptor-positive, axillary lymph-node negative breast cancer. *BMC Cancer* 2012;12:447.
- [132] Hornberger J, Cosler LE, Lyman GH. Economic analysis of targeting chemotherapy using a 21-gene RT-PCR assay in lymph-node-negative, estrogen-receptor-positive. *Early-Stage Breast Cancer* 2005;11:313–24.
- [133] Hornberger J, Chien R, Krebs K, Hochheiser L. US insurance program's experience with a multigene assay for early-stage breast cancer. *Am J Managed Care* 2011;17:E194–202.
- [134] Jahn B, Rochau U, Kurthaler C, Hubalek M, Miksad R, Sroczynski G, et al. Cost effectiveness of personalized treatment in women with early breast cancer: the application of OncotypeDX and Adjuvant! Online to guide adjuvant chemotherapy in Austria. *Springerplus* 2015;4:752.
- [135] Klang SH, Hammerman A, Liebermann N, Efrat N, Doberne J, Hornberger J. Economic implications of 21-gene breast cancer risk assay from the perspective of an Israeli-managed health-care organization. *Value Health* 2010;13:381–7.
- [136] Kondo M, Hoshi SL, Ishiguro H, Yoshibayashi H, Toi M. Economic evaluation of 21-gene reverse transcriptase-polymerase chain reaction assay in lymph-node-negative, estrogen-receptor-positive, early-stage breast cancer in Japan. *Breast Cancer Res Treat* 2008;112:175–87.
- [137] 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;127:739–49.
- [138] Lamond NW, Skedgel C, Rayson D, Lethbridge L, Younis T. Cost-utility of the 21-gene recurrence score assay in node-negative and node-positive breast cancer. *Breast Cancer Res Treat* 2012;133:1115–23.
- [139] Health Quality O. Gene expression profiling for guiding adjuvant chemotherapy decisions in women with early breast cancer: an evidence-based and economic analysis. *Ontario Health Technol Assess Ser* 2010;10:1–57.
- [140] Paulden M, Franek J, Pham B, Bedard PL, Trudeau M, Krahn M. Cost-effectiveness of the 21-gene assay for guiding adjuvant chemotherapy decisions in early breast cancer. *Value Health* 2013;16:729–39.
- [141] Smyth L, Watson G, Walsh EM, Kelly CM, Keane M, Kennedy MJ, et al. Economic impact of 21-gene recurrence score testing on early-stage breast cancer in Ireland. *Breast Cancer Res Treat* 2015;153:573–82.
- [142] Tsoi DT, Inoue M, Kelly CM, Verma S, Pritchard KI. Cost-effectiveness analysis of recurrence score-guided treatment using a 21-gene assay in early breast cancer. *Oncologist* 2010;15:457–65.
- [143] Ward S, Scope A, Rafia R, Pandor A, Harnan S, Evans P, et al. Gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management: a systematic review and cost-effectiveness analysis. *Health Technol Assess (Winchester, England)*. 2013;17:1–302.
- [144] Yamauchi H, Nakagawa C, Yamashige S, Takei H, Yagata H, Yoshida A, et al. Societal cost-effectiveness analysis of the 21-gene assay in estrogen-receptor-positive, lymph-node-negative early-stage breast cancer in Japan. *BMC Health Serv Res* 2014;14:372.
- [145] Bargallo-Rocha JE, Lara-Medina F, Perez-Sanchez V, Vazquez-Romo R, Villarreal-Garza C, Martinez-Said H, et al. Cost-effectiveness of the 21-gene breast cancer assay in Mexico. *Adv Ther* 2015;32:239–53.
- [146] Blohmer JU, Rezaei M, Kummel S, Kuhn T, Warm M, Friedrichs K, et al. Using the 21-gene assay to guide adjuvant chemotherapy decision-making in early-stage breast cancer: a cost-effectiveness evaluation in the German setting. 2013;16:30–40.
- [147] Hall PS, McCabe C, Stein RC, Cameron D. Economic evaluation of genomic test-directed chemotherapy for early-stage lymph node-positive breast cancer. *J Natl Cancer Inst* 2012;104:56–66.
- [148] Hannouf MB, Xie B, Brackstone M, Zaric GS. Cost effectiveness of a 21-gene recurrence score assay versus Canadian clinical practice in post-menopausal women with early-stage estrogen or progesterone-receptor-positive, axillary lymph-node positive breast cancer. *Pharmacoeconomics* 2014;32:135–47.
- [149] Kip M, Monteban H, Steuten L. Long-term cost-effectiveness of Oncotype DX (R) versus current clinical practice from a Dutch cost perspective. *J Comp Eff Res* 2015;4:433–45.
- [150] Nerich V, Curtit E, Bazan F, Montcuquet P, Villanueva C, Chaigneau L, et al. Economic assessment of the routine use of Oncotype DX(R) assay for early breast cancer in Franche-Comte region. *Bull Cancer* 2014;101:681–9.
- [151] Vanderlaan BF, Broder MS, Chang EY, Oratz R, Bentley TG. Cost-effectiveness of 21-gene assay in node-positive, early-stage breast cancer. *Am J Manage Care* 2011;17:455–64.
- [152] Blank PR, Filipits M, Dubsy P, Gutzwiller F, Lux MP, Brase JC, et al. Cost-effectiveness analysis of prognostic gene expression signature-based stratification of early breast cancer patients. *Pharmacoeconomics* 2015;33:179–90.
- [153] Mislick K, Schonfeld W, Bodnar C, Tong KB. Cost-effectiveness analysis of Mammostrat(R) compared with Oncotype DX(R) to inform the treatment of breast cancer. *Clinicoecon Outcomes Res* 2014;6:37–47.
- [154] Retel VP, Joore MA, van Harten WH. Head-to-head comparison of the 70-gene signature versus the 21-gene assay: cost-effectiveness and the effect of compliance. *Breast Cancer Res Treat* 2012;131:627–36.
- [155] Segui MA, Crespo C, Cortes J, Lluch A, Brosa M, Becerra V, et al. Genomic profile of breast cancer: cost-effectiveness analysis from the Spanish National Healthcare System perspective. *Expert Rev Pharmacoecon Outcomes Res* 2014;14:889–99.
- [156] Yang M, Rajan S, Issa AM. Cost effectiveness of gene expression profiling for early stage breast cancer: a decision-analytic model. *Cancer* 2012;118:5163–70.
- [157] Katz SJ, Morrow M. Addressing overtreatment in breast cancer: the doctors' dilemma. *Cancer* 2013;119:3584–8.
- [158] Gnant M, Steger GG. Fighting overtreatment in adjuvant breast cancer therapy. *Lancet* 2009;374:2029–30.

- [159] Petkov VI, Miller DP, Howlander N, Gliner N, Howe W, Schussler N, et al. Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *npj. Breast Cancer* 2016;2:16017.
- [160] Hornberger J, Alvarado MD, Rebecca C, Gutierrez HR, Yu TM, Gradishar WJ. Clinical validity/utility, change in practice patterns, and economic implications of risk stratifiers to predict outcomes for early-stage breast cancer: a systematic review. *J Natl Cancer Inst* 2012;104:1068–79.
- [161] Marrone M, Stewart A, Dotson WD. Clinical utility of gene-expression profiling in women with early breast cancer: an overview of systematic reviews. *Genet Med: Off J Am Coll Med Genet* 2015;17:519–32.
- [162] Esserman LJ, Yau C, Thompson CK, van 't Veer LJ, Borowsky AD, Hoadley KA, et al. Use of molecular tools to identify patients with indolent breast cancers with ultralow risk over 2 Decades. *Jama Oncol.* 2017.
- [163] EBCTCG. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. *Lancet.* 2015;386:1341–52.