Genomic Signatures in Luminal Breast Cancer

Julian Puppe Tabea Seifert Christian Eichler Henryk Pilch Peter Mallmann
Wolfram Malter

Department of Obstetrics and Gynecology, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany

Keywords

Breast cancer · Gene expression · Luminal breast cancer ·
Prognostic biomarker · Predictive biomarker

Abstract

Background: Breast cancer is a very heterogeneous disease and luminal breast carcinomas represent the hormone receptor-positive tumors among all breast cancer subtypes. In this context, multigene signatures were developed to gain further prognostic and predictive information beyond clinical parameters and traditional immunohistochemical markers. **Summary:** For early breast cancer patients these molecular tools can guide clinicians to decide on the extension of endocrine therapy to avoid over- and undertreatment by adjuvant chemotherapy. Beside the predictive and prognostic value, a few genomic tests are also able to provide intrinsic subtype classification. In this review, we compare the most frequently used and commercially available molecular tests (OncotypeDX[®], MammaPrint[®], Prosigna[®], EndoPredict[®], and Breast Cancer IndexSM). Moreover, we discuss the clinical utility of molecular profiling for advanced breast cancer of the luminal subtype. **Key Messages:** Multigene assays can help to de-escalate systemic therapy in early-stage breast cancer. Only the Oncotype DX[®] and MammaPrint[®] test are validated by entirely prospective and randomized phase 3 trials. More clinical evidence is needed to support the use of genomic tests in node-positive disease. Recent developments in high-throughput sequencing technology will provide further insights to understand the heterogeneity of luminal breast cancers in early-stage and metastatic disease.

© 2020 S. Karger AG, Basel

Introduction

Breast cancer is a heterogeneous disease and its clinical outcome depends on its biological subtypes, which are associated with distinct molecular features [1–3]. Immunohistochemically defined markers, in particular estrogen (ER) and progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2), and the proliferation marker Ki67, still play a major role in therapy recommendations for patients with early breast cancer [4]. About 70% of all breast tumors are of the luminal type and characterized by overexpression of ER, PR, and distinct cytokeratins [5]. Especially in luminal type early breast cancer, additional prognostic information is often required to provide patients with a reliable and effective therapy. Whereas endocrine therapy is mostly offered for women with luminal breast cancer, only a subset of patients benefits from adjuvant chemotherapy. Thus, reliable biomarkers are needed in order to identify patients with a late recurrence risk to avoid overtreatment and improve the development of de-escalation and escalation treatment strategies. Nowadays, breast cancer treatment is changing with the availability of multigene signatures. These gene signatures provide a standardized quantitative and reproducible tool to define the risk of distant recurrence for women with ER-positive and HER2-negative early breast cancer [6]. There is increasing evidence that these multigene signatures give complementary information about the clinical outcome, the benefit of che-

Julian Puppe and Tabea Seifert contributed equally to this work.

motherapy, and the identification of the molecular subtype beyond routinely obtained pathological biomarkers [2, 7]. However, there is no specific indication regarding the situations in which a multigene assay should be preferred, and the prognostic and predictive value differs between the tests. This review will focus on 5 commercially available prognostic signatures for breast cancer (Onco-typeDX[®], MammaPrint[®], Prosigna[®], EndoPredict[®], and Breast Cancer IndexSM) which are included in national and international guidelines (NCCN, ASCO, ESMO, NICE, AGO, and St. Gallen). Furthermore, we discuss the clinical utility of genomic profiling of advanced luminal breast tumors for guiding personalized therapy and immune gene expression panels to further subclassify luminal breast tumors.

Breast Cancer Molecular Subtypes and Heterogeneity of Luminal Breast Tumors

Novel molecular methods including DNA microarray analysis and RNA sequencing in combination with sophisticated bioinformatics provide novel possibilities for studying breast disease. With the development of these advanced molecular technologies, we are now able to dissect the molecular make-up of each tumor to get a deeper understanding of breast cancer heterogeneity. Characterization of breast cancer by DNA microarray identified the following 5 major subtypes by hierarchical clustering of gene expression profiles [1, 2, 7]: ER-positive/HER2-negative (luminal A and luminal B subtype), ER-negative/HER2-negative (basal subtype), and HER2-positive. Further breast carcinomas with a distinct intrinsic subtype have been identified (claudin-low [8], metaplastic [9], molecular apocrine [10], and invasive lobular carcinomas [11]). All of these subtypes are associated with a certain mRNA expression profile and clinical course and therefore considered intrinsic.

Differentiation between both luminal subtypes is important since the low-proliferating luminal A breast carcinomas are associated with a better prognosis compared to the luminal B subtype. Moreover, patients with highly endocrine sensitive tumors (e.g., high ER expression), in particular those with a luminal A low-proliferating disease, seem to derive less benefit from combined chemo-endocrine therapy versus endocrine therapy alone as shown by a retrospective analysis of some prospective trials [12, 13]. In contrast, chemotherapy should be offered to patients with more aggressive and highly proliferative luminal B breast carcinomas [14]. In clinical routine, luminal A and B disease is frequently identified by immunohistochemical (IHC) markers, especially by the proliferation marker Ki67 according to St. Gallen consensus recommendations [15, 16]. However, proliferation assessment by the use of Ki67

can differ between laboratories and the histopathological definition of luminal A and B subtypes relies on a few markers [17, 18]. The analysis of transcriptomic profiles and genomic aberrations has led to a refinement of the molecular classification by capturing the tumor biology in a more accurate and reproducible way. The most popular test to define the intrinsic subtypes is the Prosigna[®] test (PAM50 signature), which can distinguish between the molecular breast cancer subtypes based on centroids calculated for the 50 most variant genes [19]. An alternative test to the PAM50 signature is the 3-gene model [20] or MammaTyper[®], a quantitative molecular tool that measures mRNA levels of ERBB2, estrogen-sensing receptor 1 (ESR1), PGR, and MKI67 with high rates of agreement with immunohistochemistry-based subtyping of luminal Her2/neu negative breast cancer [21]. Moreover, the 80-gene molecular subtyping assay Blueprint[®] can distinguish between the 4 intrinsic subtypes [22]. Most of these tests have shown a better prognosis in luminal A versus luminal B patients, but no reliable data on missing chemotherapy from prospective trials are available so far.

Integrated analysis of genetic changes within the tumor revealed a deeper complexity [23]. Different subtypes have been linked to different copy number variations [24, 25]. The DNA copy number profile of luminal A tumors is different from that of luminal B tumors, with a more complex genomic pattern and amplifications in the FGFR1 locus [26]. Not much is known about the heterogeneity of immune gene expression patterns of luminal breast cancer since the prognosis of ER-positive breast cancer is less affected by lymphocyte content [27]. However, immune gene expression profiling of luminal tumors identified 3 immune subtypes of luminal breast tumors displaying distinct patterns of immune-related genes (with lower or higher levels of tumor-infiltrating lymphocytes or increased expression of interferon-stimulated genes and enrichment for TP53 somatic mutations) [28]. In addition, luminal A and B carcinomas consist of a mixture of different genotypes and can be further divided into 2 subgroups (diploid/CIN- and aneuploid/CIN+) based on DNA ploidy and chromosomal instability (CIN) [29, 30]. This heterogeneity influences the accuracy of diagnosis and therefore has an impact on clinical decision making. Comprehensive transcriptomic and genomic investigations of luminal breast cancers are required to gain further insight into their biological behavior [30–32].

Prognostic and Predictive Genomic Signatures in Early Luminal Breast Cancer

Over the last decade, algorithms have been developed to estimate the risk of recurrence (ROR) and survival based on a molecular gene expression signature. Patients

Table 1. Characteristics of the most common and commercially available genomic signatures

	Oncotype DX®	MammaPrint®	EndoPredict® (EP)	Prosigna® (PAM50, ROR)	Breast Cancer Index®
Manufacturer	Genomic Health	Agendia NV	Myriad Genetics	NanoString Technologies	Biotheranostics
Genes	21 genes (16 cancer-related + 5 reference genes)	70 genes	12 genes (EP) (8 cancer-related + 4 reference genes)	50 genes (+5 reference genes)	7 genes (HOXB13/IL17BR ratio + 5 genes)
Test method	RT-PCR	RT-PCR or RNA microarray	RT-PCR	RT-PCR (nCounter®)	RT-PCR
Central lab	Yes	Yes	No	No	Yes
Type of tissue	FFPE	FFPE or fresh tissue	FFPE	FFPE	FFPE
Classification	RS 0–100: Low risk (0–10) Intermediate risk (11–25) High risk (>25 in women aged <50 years, >15 and high clinical risk or >21, independently of the clinical risk) [38, 42]	MammaPrint Index: Low risk High risk	EP molecular score 0–15: Low risk (<5) High risk (>5) EPclin score (with tumor size and nodal status): Low risk (<3.3) High risk (≥3.3)	ROR 0–100: Low risk (<40) Intermediate risk (41–60) High risk (61–100)	Molecular grade index 0–10: Low risk (<5) Intermediate risk (5–6.3) High risk (>6.3)
Clinical applicability	Prognostic, endocrine treated, HR+, HER2-, N-/+	Prognostic, age <70 years, HR+, HER2-, N-/+	Prognostic, endocrine treated, postmenopausal, HR+, HER2-, N-/+	Prognostic, endocrine treated, postmenopausal, HR+, HER2-, N-/+	Prognostic, endocrine treated, HR+, HER2-, N-/+
Prognosis after 5 years (late recurrence)	Yes	Not separately shown	Yes	Yes	Yes
Predictive value (chemotherapy)	Yes	Yes	No	No	No
Predictive value (endocrine therapy)	Yes	No	No	Yes	Yes (extended endocrine therapy)
Molecular subtyping	No	Yes (BluePrint®)	No	Yes	No
Clinical studies	NSABP B14 NSABP B20 SWOG 8814 TransATAC (retrospective) TAILORx WSG-PlatB (prospective) RxPonder WSG-ADAPT (prospective, ongoing)	TRANSBIG (retrospective) RASTER MINDACT (prospective)	GEICAM 9906 ABCSG6 ABCSG8 (retrospective)	ABCS8 ATAC (retrospective)	TransATAC, Stockholm trials (retrospective)
Guideline recommendations (chemotherapy)	NCCN (1 for N0, 2A for N+) ASCO (N0, strong) NICE St. Gallen/ESMO IQWiG AGO + (adj.)	NCCN (1 for N0 and 1–3 N+) ASCO (N0 strong, 1–3 N+ moderate) St. Gallen ESMO AGO + (adj.)	NCCN (2A for N0 and 1–3 N+) ASCO (N0, moderate) NICE St. Gallen ESMO AGO + (adj.)	NCCN (2A for N0 and 1–3 N+) ASCO (N0, strong) NICE St. Gallen ESMO AGO + (adj.)	NCCN (2A) ASCO (N0, moderate) AGO +/- (NACT)
Adapted from Gluz et al. [63], Vieira and Schmitt [64], and Gynäkologische Onkologie e.V. (AGO) [96]. adj., adjuvant.					

were classified by distinct gene expression profiles and stratified according to their clinical outcome [33, 34]. These assays should detect the genes of interest (analytic validity) in central, but also decentralized, laboratories. For this reason, formalin-fixed paraffin embedded (FFPE) tissue samples are preferred over fresh frozen material. Moreover, genetic tests should classify a population into different groups of patients with another clinical outcome (i.e., recurrence-free survival or overall survival) (clinical validity). The clinical value of a test must be demonstrated in clinical trials, ideally in prospective studies [34]. In the following sections, we describe the most clinically used multigene signatures (Oncotype DX[®], MammaPrint[®], Prosigna[®], EndoPredict[®], and Breast Cancer IndexSM) which are commercially available (Table 1).

Oncotype DX[®]/Recurrence Score

Oncotype DX[®] (Genomic Health, Redwood, CA, USA) is the most frequently used breast cancer multigene test. This test consists of a 21-gene signature (16 cancer and 5 reference genes) and it is based on an RT-PCR of RNA extracted from FFPE tissue [35]. The 5-year or 10-year risk of distant relapse is stratified by recurrence score (RS) into 3 risk groups (i.e., low risk, RS <18; intermediate risk, RS 18–31; and high risk, RS >10). These RS cut-off scores were based on retrospective trials and they were modified by the latest clinical trial [36–38]. The test was initially validated in tamoxifen-treated node-negative (N0) ER-positive patients from the NSABP B14 clinical trial [35]. The chemotherapy benefit of the 21-gene RS for was shown by retrospective analyses of the NSABP B14 and 20 studies and the SWOG 8814 trial, especially for patients with an RS >31 [12, 35, 39]. The SWOG 8814 trial demonstrated that the RS was prognostic in postmenopausal breast cancer patients with HR-positive, HER2-negative, and node-positive disease [12]. The TransATAC study evaluated the RS in postmenopausal N0 and N+ hormone receptor (HR)-positive patients treated with endocrine therapy [40].

The TAILORx study was a prospective phase III trial and included HR-positive, HER-2-negative, and node-negative patients [38, 41]. The treatment choice was stratified by RS. Patients with an RS between 11 and 25 were randomized to endocrine therapy alone versus adjuvant chemo- and endocrine therapy. First data from the TAILORx study showed that women in the low RS group (RS <11) had a very low risk of recurrence (ROR) after 5 years (<10%) with endocrine therapy alone [36]. Recently, the final results of the TAILORx study were published and they clarified the benefit of chemotherapy for the intermediate risk group [38]. In this study the interaction between chemotherapy and clinical parameters in the randomized arms was analyzed and a significant interaction

was observed between age and chemotherapy benefit. The study suggests that chemotherapy may be spared in women aged >50 years with an RS between 11 and 25. Subgroups analysis showed that women aged ≤50 years with an RS of 16–20 and an RS of 21–25 might benefit from chemotherapy with respect to both locoregional and distant recurrences. A recent exploratory analysis of premenopausal patients, aged ≤50 years, from the TAILORx study included a clinical-risk stratification (low or high risk based on tumor size and histologic grade). It confirmed a potential chemotherapy benefit for younger women with an RS between 16 and 20 and a high clinical risk as well as for young women with an RS between 21 and 25, independently of their clinical risk [42]. Thus, the integration of clinicopathological factors could provide prognostic but not predictive information regarding a chemotherapy benefit when added to the 21-gene RS [42, 43].

Oncotype DX[®] was established as prognostic and predictive test for HR+/HER2-negative and node-negative breast cancer patients. However, its clinical value in node-positive (N+) patients has not been elucidated. In the prospective phase III PlanB trial (West German Study Group) chemotherapy could be omitted in patients with an RS ≤11 in node-negative and node-positive disease with up to 3 positive lymph nodes. The WSG PlanB study demonstrated that 17% of clinically high-risk patients with a low RS had an excellent 5-year disease-free survival rate of 94% without receiving chemotherapy [44, 45]. However, results from the WSG PlanB and TAILORx studies might indicate overtreatment of patients with an intermediate RS. Recently, the RS was compared with clinical parameters of 4,695 German breast cancer patients tested in routine clinical practice. This real-world data analysis showed that 83% of node-negative and 90% of node-positive patients had a low or intermediate RS, indicating that chemotherapy may not be beneficial in most of these patients [46].

Data from the ongoing prospective and randomized RxPONDER (ClinicalTrials.gov identifier: NCT-01272037) and WSG-ADAPT trials (ClinicalTrials.gov identifier: NCT01817452) will provide further evidence of the chemotherapy benefit and help to determine the optimal RS cut-off values for chemotherapy omission in ER+/HER2- and node-positive early breast cancer patients. Moreover, the benefit of chemotherapy in postmenopausal women with node-negative disease and an RS between 25 and 31 still needs to be clarified due to the shift of RS cut-offs. The ongoing phase III WSG-ADAPT-cycle trial will help to elucidate the effect of endocrine therapy plus the CDK4/6 inhibitor ribociclib versus a chemotherapy benefit for this intermediate risk group (ClinicalTrials.gov identifier: NCT04055493).

MammaPrint®

MammaPrint® (Agendia NV, Amsterdam, The Netherlands) is a 70-gene signature first developed by The Netherlands Cancer [47]. It is a prognostic test that classifies breast cancer patients into high- and low-risk group [48]. Initially, the test required fresh-frozen tissue but it is now also optimized for FFPE tissue samples [49]. The test was further validated in node-negative patients in the RASTER trial [50]. Clinical utility in early-stage luminal breast cancer was recently confirmed by the prospective randomized MINDACT trial [50, 51]. In that phase III study 6,693 women with early-stage breast cancer (lymph node negative or 1–3 lymph node positive) were enrolled. The genomic ROR was analyzed by MammaPrint® and the clinical risk of relapse was defined using Adjuvant! Online. Subsequently, patients were divided into 4 groups according to their clinical (C) and genomic (G) risk. The groups with discrepant genomic and clinical risks (C-low/G-high and C-high/G-low) were randomized to the endocrine alone arm versus adjuvant chemo- and endocrine therapy. The primary endpoint of the MINDACT study was to determine whether C-high/G-low patients who did not receive chemotherapy were low risk as defined by having a 5-year distant metastasis-free survival (DMFS) $\geq 92\%$ including a 95% CI. With a 5-year DMFS of 94.7% (95% CI 92.5–96.2) in the C-high/G-low group, the primary objective was achieved [51]. The data suggested a slightly worse prognosis for C-high/G-low patients who did not receive chemotherapy. This difference was statistically significant for disease-free survival but not for DMFS or overall survival. A longer follow-up of the MINDACT study is now available and continues to meet the primary endpoint at 5 years, with an updated DMFS of 95.1% (95% CI 93.1–96.6) in chemotherapy-untreated C-high/G-low patients [52]. Overall, the 70-gene signature does not support a predictive utility but is the only multigenomic assay with prospective level 1a evidence for determining the prognosis in clinically high-risk node-negative and node-positive patients.

The WSG-PRIME study prospectively evaluated the impact of the MammaPrint® combined with the Blueprint® assay on clinical therapy decisions and concluded that the test results strongly impacted therapy decisions, with a high adherence to the genetically determined risk [22]. The Blueprint® test is an 80-gene assay that enables breast cancer subclassification into a low-risk luminal type, a high-risk luminal type, a HER-2 type, and a basal-like type [53]. Interestingly, molecular subtyping by Blueprint® identified more luminal A tumors than analyzed by classical IHC markers. Blueprint® and PAM50 signature show a great similarity between both gene sets. However, it is still unclear which methodology is better for subclassification of breast cancer molecular subtypes.

Prosigna®/PAM50/Risk of ROR

The Prosigna® test (NanoString Technologies, Seattle, WA, USA) is a multigene signature of the second generation. The test includes 50 genes based on NanoString nCounter® technology and it is approved to estimate distant recurrence in early ER-positive breast cancer with maximum 3 positive lymph nodes in postmenopausal women treated with endocrine therapy alone [54, 55]. The current available PAM50 profile can be done from FFPE tissue samples by local pathology laboratories and provides an ROR score. The test results are combined with nodal status and tumor size to establish the ROR score. The ROR score is correlated with the 10-year probability of distant recurrence, with risk groups categorized as low (< 10%), intermediate (10–20%), and high (>20%) ROR [35, 56]. It can also distinguish between the molecular subtypes of breast cancer (i.e., luminal A, luminal B, HER2-enriched, and basal-like). The gene expression profile is compared with each of the 4 molecular subtypes to determine the degree of similarity [19]. The ABCG-8 study and the TransATAC trials evaluated this molecular test in endocrine-treated patients with ER-positive, node-negative disease [40, 55]. In a small retrospective analysis of the TransATAC trial the Prosigna® test was compared with Oncotype DX®. More patients were stratified as high risk and fewer as intermediate risk by ROR than by RS [6]. In addition, the ROR provides more prognostic information with a better outcome in low-risk endocrine-treated patients compared to the 21-gene assay [6]. The prognostic value of the Prosigna® test has already been demonstrated, but prospective data of the predictive value is currently missing. OPTIMA is a large ongoing prospective trial that is validating the clinical utility and cost-effectiveness of test-guided chemotherapy decisions in node-positive early breast cancer by using the Prosigna® (PAM50) test [57].

EndoPredict® (EPclin)

EndoPredict® (Myriad Genetics, Inc.) is a 12-gene signature and calculates a risk score called the endopredict (EP) score. The EP score can be combined with tumor size and nodal status to obtain the more comprehensive risk score EPclin. The test can be used to guide therapy decision for chemo- and extended endocrine therapy [58]. This multigene signature was evaluated in the GEICAM trial as an independent prognostic parameter in node-positive, ER+/HER2– breast cancer patients for adjuvant chemo- and endocrine therapy [59]. Analysis of the ABCSG6 and ABCSG8 trial indicated that the EP and EPclin score can provide additional information about the risk of distant recurrence in patients with node-negative and node-positive disease, independently of clinicopathologic parameters [58]. The EPclin score may also be used to guide physicians in their decision-making process

to apply systemic chemotherapy in postmenopausal patients with ER-positive, HER2-negative, node-negative breast tumors. For node-positive patients, clinical use of the EndoPredict® test is not recommended so far. It was also suggested that this test might predict the benefit of extended endocrine therapy due to the fact that the genes involved in this multigene signature are mainly associated with ER signaling. Prospective studies are warranted to confirm this hypothesis.

Breast Cancer Index®

Breast Cancer Index® (BCI; Biotheranostics, Inc.) is based on the HOXB13:IL17BR gene expression ratio and 5 proliferation genes measured by RT-PCR. This combined signature is known as the molecular grade index and it predicts the risk of both early and late (>5 years) distant recurrences and reports the likelihood of a benefit from endocrine therapy in postmenopausal patients with ER-positive, lymph node-negative breast cancer [60]. The prognostic utility of the test was further validated in the TransATAC and the Stockholm trials [61, 62]. The clinical evidence of the extension of adjuvant endocrine therapy beyond 5 years and the use of BCI in luminal node-positive breast cancer is still insufficient.

Comparison of Different Multigene Signatures

The utility of different multigene signatures has been proven in multiple large trials, indicating that mRNA-based multigenomic assays are useful in aiding adjuvant therapy decision making in early luminal breast carcinomas [63–65]. However, these genomic signatures are quite heterogeneous and 2 tests performed on the same sample might lead to different results. As demonstrated by the prospective phase III OPTIMA prelim trial, the concordance between different assays seems to be only moderate [66]. In this study, several multigene signatures were compared including Oncotype DX®, MammaPrint® and Prosigna®, MammaTyper® and the IHC4 test. By using different molecular tests on the same tumor tissue, you might also get various recommendations for treatment decisions. The concordance of MammaPrint® and Oncotype DX® in ER-positive breast cancer patients from the same risk category was only 77% [66]. Recently, the PROMIS trial showed that intermediate-risk patients, as classified by RS, had a low-risk result in 45% and a high-risk result in 55% of cases when reanalyzed with MammaPrint® [67]. Use of the MammaPrint® led to a change in physicians' treatment decisions in about 34% of all cases.

In contrast to the Endopredict® and Prosigna® tests the Oncotype DX® and MammaPrint® assays are currently only available as a central laboratory service, which

limits their clinical use and raises concerns about the reproducibility and calculation algorithm of these multigene signatures. Recently, the MammaPrint® and BluePrint® RNA sequencing test was found to be equivalent to the clinically validated microarray test [68]. It was also demonstrated that the MammaPrint RNA-sequencing test can be used as a decentralized platform with results equivalent to those derived from the predicated diagnostic device [68, 69]. Independent intra-assay reproducibility was also shown for the Prosigna® and EndoPredict® assays [70, 71]. In a similar approach another group tried to compare the performance of the Oncotype DX® RS with a customized RT² profiler multiplex RT-PCR covering the 21-gene panel to recapitulate the transcription profile as claimed by Genomic Health [72]. Unexpectedly, they could not establish a correlation between the 2 assays, indicating that the presented data cannot be reproduced by others independent methods. This variation in risk stratification might have been caused by differences in the study cohorts, tumor biology, the used molecular techniques, or undisclosed original data algorithms. The TAILORx study did not include a control group stratified by clinical and histopathological diagnostics without the RS result. Due to the fact that this control group was missing and the Oncotype DX® was an inclusion criterion, it remains controversial whether the risk stratification benefits exclusively from the 21-gene assay in addition to clinical parameters [72]. On the other hand, when the TAILORx study was started in the USA, all patients with a tumor size >1 cm were candidates for chemotherapy and a large meta-analysis of taxane- and anthracycline-based regimens could not identify nodal status, tumor size, or grading as predictive clinical markers [73]. This issue might be clarified by decentralized retesting as planned with the Oncotype DX® adapted Biocartis Idylla System.

A comparative study of 6 prognostic signatures including Oncotype DX®, Prosigna®, BCI, EPclin, IHC4, and the Clinical Treatment Score for ER-positive breast cancer showed that the Prosigna® test, BCI, and EPclin signatures provided superior prognostic accuracy in node-negative breast cancer patients, whereas BCI and EPclin were better in node-positive breast disease [54]. Two retrospective studies analyzed the difference in oncological decision making for the treatment of ER+/HER2– early breast cancer with and without the results of the Oncotype DX® or EndoPredict® multigenomic assays [74, 75]. It seems that especially inexperienced physicians may profit from the additional information of the genomic signatures to avoid overtreatment.

These genomic tests have the capacity to revolutionize clinical practice. However, extensive gene expression profiling is having an increasing economic impact on the health care system [76]. In a budget impact analysis dif-

ferent multigene signatures including Oncotype DX[®], MammaPrint[®], Prosigna[®] and EndoPredict[®] were compared. Here, Oncotype DX[®] was the only genomic signature that was estimated to reduce costs versus standard care in Germany [77]. With the recent TAILORx findings the German Institute for Quality and Efficiency in Health Care (IQWiG) had enough evidence to recommend the use of the 21-gene assay in node-negative ER-positive early breast cancer [65]. Therefore, reimbursement of Oncotype DX[®] becomes easier in clinical practice.

Molecular Profiling of Advanced Luminal Breast Cancer

While the clinical utility of prognostic multigene signatures has been established in early breast cancer, the role in metastatic disease is less explored. A recent study used the PAM50-signature to compare the intrinsic subtype in paired primary and metastatic tissue [78]. It was shown that the intrinsic subtype was mostly maintained during metastatic progression. However, luminal A/HER2-negative tumors can also acquire a luminal B or HER2-enriched profile in some cases, reflecting tumor evolution [78]. This is in line with the observed discordance of *HER2*-gene amplification [79] and ER/PR receptor status by IHC in primary versus metastatic tissue [80]. Another study evaluated the PAM50 signature for postrelapse survival prognostication according to first-line treatment for metastatic disease and identified the ROR score from the primary tumor as independent prognostic factor of postrelapse survival [81]. Despite these promising results, the clinical utility of multigene signatures should be validated in larger independent cohorts of advanced breast cancer.

Of note, these multigene tests cannot provide information about the mutational burden, which might also have an impact on breast cancer prognosis. Therefore, analysis of the complete spectrum of cancer mutations by whole genome sequencing might also be crucial to predict a druggable target for a tailored therapy. In this context Foundation One CDx (Foundation Medicine, Cambridge, MA, USA, and Roche, Basel, Switzerland) is an FDA-approved diagnostic tool that provides a comprehensive genomic profile matched with potential targeted therapies. Up to now, little data supports large-scale genomic testing of advanced breast tumors and the results of the first clinical trials have been disappointing since only 11% have actually been treated according to their molecular tumor profile [82]. Therefore, the integration of genomic test panels in clinical routine remains controversial and should be reserved for difficult-to-treat cases.

PIK3CA is recurrently mutated in about 30–40% of ER+ breast tumors and it is therefore one of the most fre-

quent genetic aberrations in luminal breast cancer [55]. This suggests a dependency of ER+ breast cancer cells on the PI3K/AKT/mTOR signaling pathway and provides a promising therapeutic target [83]. Alpelisib is a p110 α isoform specific oral PIK3CA inhibitor, and results from the SOLAR I trial showed a benefit from treatment with Alpelisib in combination with fulvestrant in patients with advanced breast cancer carrying *PIK3CA* mutations [84]. These *PIK3CA* mutations remain almost stable between the primary tumor and metastases, indicating that a biopsy of the primary tumor site is sufficient for therapy indication [83]. Therefore, routine molecular testing for *PIK3CA* mutations should be implemented for the treatment of ER+ metastatic disease.

Mutations of *ESR1* have been shown to emerge in metastases and they have been recognized as prognostic and predictive biomarkers of the response to endocrine therapy in luminal breast cancer [85, 86]. Clinical phase 1 trials are currently testing several oral selective estrogen receptor degrader (SERD) including G1T48 (ClinicalTrials.gov identifier: NCT03455270) in metastatic endocrine resistant luminal breast cancer harboring *ESR1* mutations [86]. Despite these novel developments the clinical integration of routine testing for *ESR1* mutations needs further validation.

About 10% of all breast cancer patients carry a *BRCA1* or *BRCA2* germline mutation, which is frequently associated with a triple-negative phenotype [87]. However, a substantial proportion of *BRCA*-mutated breast cancers are ER+ and display a luminal subtype, and the majority of them are *BRCA2* mutation carriers (luminal A: *BRCA1*: 9% and *BRCA2*: 35%; luminal B: *BRCA1*: 21% and *BRCA2*: 40%) [88]. This study also showed that *BRCA*-mutated breast tumors of the luminal A subtype have a better prognosis. For the treatment of metastatic ER-positive breast cancer CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) are standard of care [89]. Less is known about the effect of endocrine therapy in patients with metastatic ER-positive and *BRCA*-mutated breast cancer [90]. In this context, the PARP inhibitors olaparib and talazoparib were recently approved for the treatment of metastatic breast cancer in patients with *BRCA* germline mutations [91, 92]. However, the optimal therapy sequence of CDK4/6 and PARP inhibitors in ER-positive *BRCA1/2*-mutated advanced breast cancer remains unclear. Further studies should investigate the impact of molecular intrinsic subtypes on survival and treatment response in *BRCA*-mutated breast cancer patients. More potential targets for the treatment of advanced luminal carcinomas are under development and include androgen receptors [93]. Combining the BCL2 inhibitor venetoclax with tamoxifen to target apoptosis could represent another new strategy for the treatment of ER-positive metastatic breast cancer [94]. Moreover, the implementa-

tion of high-throughput techniques to analyze tumor heterogeneity between metastasis or single tumor cells and free circulating DNA or micro-RNA profiling will pave the way to more targeted gene expression signatures in this tumor subtype.

Conclusions

Multigene assays can help physicians to guide treatment in early-stage breast cancer by selecting the use of adjuvant chemotherapy. Multiple retrospectively analyzed studies support the clinical utility of these genomic signatures. Only the Oncotype DX[®] and MammaPrint[®] tests are supported by entirely prospective and randomized phase 3 trials. These multigene signatures are not interchangeable and will give different information depending on the clinical setting. The 21-gene assay was validated with level 1a evidence to predict the benefit from adjuvant chemotherapy in node-negative ER-positive HER2-negative early breast cancer. In contrast, MammaPrint[®] is the only multigene signature with level 1a evidence for prognosis determination in node-positive disease. However, NCCN guidelines recommend the use of a 70-gene signature as a prognostic option only for patients with a high clinical risk and up to 1–3 positive nodes [95]. Despite the results of the WSG-PlanB study indicating that genomically low-risk patients have a similar outcome in node-negative and node-positive cases, more clinical evidence is needed to clarify the use of multigene tests in the node-positive setting. Moreover, the clinician should be aware of the clinical utility and limitations when applying such a genetic test.

The prognostic and predictive role of multigene signatures in advanced luminal breast cancer is poorly under-

stood. Moreover, tumor heterogeneity influences the diagnosis and makes clinical decision making more complex. Therefore, profound genetic characterization of luminal breast cancers in early-stage and metastatic disease should provide further insight into their biological behavior to guide treatment decisions.

In summary, genomic signatures will be further implemented in the clinic to individualize systemic treatment by reducing the rate of adjuvant chemotherapy for the treatment of luminal breast cancer.

Statement of Ethics

No ethical approval was required for this review article.

Conflict of Interest Statement

Wolfram Malter received honoraria from Genomic Health (advisory board), Pfizer (advisory board), Novartis (advisory board), NanoString, Celgene, and Roche. Tabea Seifert, Christian Eichler, Henryk Pilch, Peter Mallmann, and Julian Puppe have no conflict of interests to declare.

Funding Sources

No funding was received for this study.

Author Contributions

Tabea Seifert and Julian Puppe substantially contributed to the conception of the article, review of literature, and drafting of this paper. All of the authors critically revised this work and approved the final version to be published.

References

- 1 Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA*. 2003 Jul;100(14):8418–23.
- 2 Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature*. 2000 Aug;406(6797):747–52.
- 3 Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al.; METABRIC Group. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*. 2012 Apr;486(7403):346–52.
- 4 Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsy P, Loibl S, et al.; St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2017. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. *Ann Oncol*. 2017 Aug;28(8):1700–12.
- 5 Creighton CJ. The molecular profile of luminal B breast cancer. *Biologics*. 2012;6:289–97.
- 6 Dowsett M, Sestak I, Lopez-Knowles E, Sidhu K, Dunbier AK, Cowens JW, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol*. 2013 Aug;31(22):2783–90.
- 7 Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lønning PE, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA*. 2001;98(19):10869–74.
- 8 Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JL, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Res*. 2010;12(5):R68.
- 9 Weigelt B, Ng CK, Shen R, Popova T, Schizas M, Natrajan R, et al. Metaplastic breast carcinomas display genomic and transcriptomic heterogeneity. *Mod Pathol*. 2015 Mar;28(3):340–51.
- 10 Farmer P, Bonnefoi H, Becette V, Tubiana-Hulin M, Fumoleau P, Larsimont D, et al. Identification of molecular apocrine breast tumours by microarray analysis. *Oncogene*. 2005 Jul;24(29):4660–71.
- 11 Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, et al.; TCGA Research Network. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. *Cell*. 2015 Oct;163(2):506–19.

- 12 Albain KS, Barlow WE, Shak S, Hortobagyi GN, Livingston RB, Yeh IT, et al.; Breast Cancer Intergroup of North America. 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 Jan;11(1):55–65.
- 13 Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res*. 2010 Nov;16(21):5222–32.
- 14 Coates AS, Winer EP, Goldhirsch A, Gelber RD, Gnant M, Piccart-Gebhart M, et al.; Panel Members. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. *Ann Oncol*. 2015 Aug;26(8):1533–46.
- 15 Lüftner D, Bauerfeind I, Braun M, Brucker SY, Fasching PA, Felberbaum R, et al. Treatment of Early Breast Cancer Patients: Evidence, Controversies, Consensus: Focusing on Systemic Therapy - German Experts' Opinions for the 16th International St. Gallen Consensus Conference (Vienna 2019). *Breast Care (Basel)*. 2019 Oct;14(5):315–24.
- 16 Balic M, Thomssen C, Würlstein R, Gnant M, Harbeck N. St. Gallen/Vienna 2019: A Brief Summary of the Consensus Discussion on the Optimal Primary Breast Cancer Treatment. *Breast Care (Basel)*. 2019 Apr;14(2):103–10.
- 17 Varga Z, Diebold J, Dommann-Scherrer C, Frick H, Kaup D, Noske A, et al. How reliable is Ki-67 immunohistochemistry in grade 2 breast carcinomas? A QA study of the Swiss Working Group of Breast- and Gynecopathologists. *PLoS One*. 2012;7(5):e37379.
- 18 Maranta AF, Broder S, Fritzsche C, Knauer M, Thürlimann B, Jochum W, et al. Do YOU know the Ki-67 index of your breast cancer patients? Knowledge of your institution's Ki-67 index distribution and its robustness is essential for decision-making in early breast cancer. *Breast*. 2020 Jun;51:120–6.
- 19 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 Mar;27(8):1160–7.
- 20 Prat A, Parker JS, Fan C, Perou CM. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. *Breast Cancer Res Treat*. 2012 Aug;135(1):301–6.
- 21 Finsterbusch K, Decker T, van Diest PJ, Focke CM. Luminal A versus luminal B breast cancer: MammaTyper mRNA versus immunohistochemical subtyping with an emphasis on standardised Ki67 labelling-based or mitotic activity index-based proliferation assessment. *Histopathology*. 2020 Apr;76(5):650–60.
- 22 Wuerstlein R, Kates R, Gluz O, Grischke EM, Schem C, Thill M, et al.; WSG-PRIME investigators in Germany, Austria, Switzerland. Strong impact of MammaPrint and BluePrint on treatment decisions in luminal early breast cancer: results of the WSG-PRIME study. *Breast Cancer Res Treat*. 2019 Jun;175(2):389–99.
- 23 Hofree M, Shen JP, Carter H, Gross A, Ideker T. Network-based stratification of tumor mutations. *Nat Methods*. 2013 Nov;10(11):1108–15.
- 24 Bergamaschi A, Kim YH, Wang P, Sørli T, Hernandez-Boussard T, Lonning PE, et al. Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. *Genes Chromosomes Cancer*. 2006 Nov;45(11):1033–40.
- 25 Ciriello G, Sinha R, Hoadley KA, Jacobsen AS, Reva B, Perou CM, et al. The molecular diversity of Luminal A breast tumors. *Breast Cancer Res Treat*. 2013 Oct;141(3):409–20.
- 26 Chin K, DeVries S, Fridlyand J, Spellman PT, Roydasgupta R, Kuo WL, et al. Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. *Cancer Cell*. 2006 Dec;10(6):529–41.
- 27 Chung YR, Kim HJ, Jang MH, Park SY. Prognostic value of tumor infiltrating lymphocyte subsets in breast cancer depends on hormone receptor status. *Breast Cancer Res Treat*. 2017 Feb;161(3):409–20.
- 28 Zhu B, Tse LA, Wang D, Koka H, Zhang T, Abubakar M, et al. Immune gene expression profiling reveals heterogeneity in luminal breast tumors. *Breast Cancer Res*. 2019 Dec;21(1):147.
- 29 Network CG; Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012 Oct;490(7418):61–70.
- 30 Yanagawa M, Ikemoto K, Kawauchi S, Furuya T, Yamamoto S, Oka M, et al. Luminal A and luminal B (HER2 negative) subtypes of breast cancer consist of a mixture of tumors with different genotype. *BMC Res Notes*. 2012 Jul;5(1):376.
- 31 Tishchenko I, Milioli HH, Riveros C, Moscato P. Extensive Transcriptomic and Genomic Analysis Provides New Insights about Luminal Breast Cancers. *PLoS One*. 2016 Jun;11(6):e0158259.
- 32 Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al.; Oslo Breast Cancer Consortium (OSBREAC). The landscape of cancer genes and mutational processes in breast cancer. *Nature*. 2012 May;486(7403):400–4.
- 33 Hayes DF. Clinical utility of genetic signatures in selecting adjuvant treatment: risk stratification for early vs. late recurrences. *Breast*. 2015 Nov;24 Suppl 2:S6–10.
- 34 Kwa M, Makris A, Esteva FJ. Clinical utility of gene-expression signatures in early stage breast cancer. *Nat Rev Clin Oncol*. 2017 Oct;14(10):595–610.
- 35 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 Dec;351(27):2817–26.
- 36 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 Nov;373(21):2005–14.
- 37 Sparano JA. A 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2016 Apr;374(14):1387.
- 38 Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2018 Jul;379(2):111–21.
- 39 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 Aug;24(23):3726–34.
- 40 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 Apr;28(11):1829–34.
- 41 Sparano JA. TAILORx: trial assigning individualized options for treatment (Rx). *Clin Breast Cancer*. 2006 Oct;7(4):347–50.
- 42 Sparano JA, Gray RJ, Ravdin PM, Makower DF, Pritchard KI, Albain KS, et al. Clinical and Genomic Risk to Guide the Use of Adjuvant Therapy for Breast Cancer. *N Engl J Med*. 2019 Jun;380(25):2395–405.
- 43 Buus R, Sestak I, Kronenwett R, Denkert C, Dubsky P, Krappmann K, et al. Comparison of EndoPredict and EPclin With Oncotype DX Recurrence Score for Prediction of Risk of Distant Recurrence After Endocrine Therapy. *J Natl Cancer Inst*. 2016 Jul;108(11):djw149.
- 44 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 Jul;34(20):2341–9.
- 45 Nitz U, Gluz O, Clemens M, Malter W, Reimer T, Nuding B, et al.; West German Study Group PlanB Investigators. West German Study PlanB Trial: Adjuvant Four Cycles of Epirubicin and Cyclophosphamide Plus Docetaxel Versus Six Cycles of Docetaxel and Cyclophosphamide in HER2-Negative Early Breast Cancer. *J Clin Oncol*. 2019 Apr;37(10):799–808.
- 46 Walter VP, Taran FA, Wallwiener M, Bauer A, Grischke EM, Walter CB, et al. Distribution of the 21-Gene Breast Recurrence Score in Patients with Primary Breast Cancer in Germany. *Geburtshilfe Frauenheilkd*. 2020 Jun;80(6):619–27.
- 47 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 Jan;415(6871):530–6.
- 48 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 Dec;347(25):1999–2009.
- 49 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 Feb;6(2):e17163.

- 50 Drukker CA, Bueno-de-Mesquita JM, Retèl VP, van Harten WH, van Tinteren H, Wesselink J, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. *Int J Cancer*. 2013 Aug; 133(4):929–36.
- 51 Cardoso F, van't Veer LJ, Bogaerts J, Slaets L, Viale G, Delaloge S, et al.; MINDACT Investigators. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med*. 2016 Aug;375(8):717–29.
- 52 Cardoso F, Veer Lvt, Poncet C, Cardozo JL, Delaloge S, Pierga J-Y, Vuylsteke P, Brain E, Viale G, Kuemmel S, Rubio IT, Zoppoli G, Thompson AM, Matos E, Zaman K, Hilbers F, Dudek-Perić A, Meulemans B, Piccart-Gebhart MJ, Rutgers EJ. MINDACT: Long-term results of the large prospective trial testing the 70-gene signature MammaPrint as guidance for adjuvant chemotherapy in breast cancer patients. *J Clin Oncol*. 2020;38:506.
- 53 Krijgsman O, Roepman P, Zwart W, Carroll JS, Tian S, de Snoo FA, et al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. *Breast Cancer Res Treat*. 2012 May;133(1):37–47.
- 54 Sestak I, Buus R, Cuzick J, Dubsy P, Kronenwett R, Denkert C, et al. Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Oncol*. 2018 Apr;4(4):545–53.
- 55 Gnant M, Filipits M, Greil R, Stoeger H, Rudas M, Bago-Horvath Z, et al.; Austrian Breast and Colorectal Cancer Study Group. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol*. 2014 Feb;25(2):339–45.
- 56 Filipits M, Nielsen TO, Rudas M, Greil R, Stöger H, Jakesz R, et al.; Austrian Breast and Colorectal Cancer Study Group. 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 Mar;20(5):1298–305.
- 57 Stein R, Makris A, Hughes-Davies L, Macpherson I, Marshall A, Campbell A, et al. OPTIMA: a prospective randomized trial to validate the clinical utility and cost-effectiveness of gene expression test-directed chemotherapy decisions in high clinical risk early breast cancer. *Eur J Surg Oncol*. 2016;42:PS229.
- 58 Filipits M, Rudas M, Jakesz R, Dubsy P, Fitzal F, Singer CF, et al.; EP Investigators. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res*. 2011 Sep;17(18):6012–20.
- 59 Martin M, Brase JC, Calvo L, Krappmann K, Ruiz-Borrego M, Fisch K, et al. Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2-breast cancer patients: results from the GEICAM 9906 trial. *Breast Cancer Res*. 2014 Apr; 16(2):R38.
- 60 Sgroi DC, Carney E, Zarrella E, Steffel L, Binns SN, Finkelstein DM, et al. Prediction of late disease recurrence and extended adjuvant letrozole benefit by the HOXB13/IL17BR biomarker. *J Natl Cancer Inst*. 2013 Jul;105(14): 1036–42.
- 61 Sgroi DC, Sestak I, Cuzick J, Zhang Y, Schnabel 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 Oct;14(11):1067–76.
- 62 Jerevall PL, Ma XJ, Li H, Salunga R, Kesty NC, Erlander MG, et al. Prognostic utility of HOXB13:IL17BR and molecular grade index in early-stage breast cancer patients from the Stockholm trial. *Br J Cancer*. 2011 May; 104(11):1762–9.
- 63 Gluz O, Hofmann D, Würstlein R, Liedtke C, Nitz U, Harbeck N. Genomic profiling in luminal breast cancer. *Breast Care (Basel)*. 2013 Dec;8(6):414–22.
- 64 Vieira AF, Schmitt F. An update on breast cancer multigene prognostic tests: emergent clinical biomarkers. *Front Med (Lausanne)*. 2018 Sep;5:248.
- 65 IQWiG. D14-01: biomarker-based tests to support the decision for or against adjuvant systemic chemotherapy in primary breast cancer. Cologne: IQWiG; 2020.
- 66 Bartlett JM, Bayani J, Marshall A, Dunn JA, Campbell A, Cunningham C, et al.; OPTIMA TMG. Comparing Breast Cancer Multiparameter Tests in the OPTIMA Prelim Trial: No Test Is More Equal Than the Others. *J Natl Cancer Inst*. 2016 Apr;108(9):djw050.
- 67 Tsai M, Lo S, Audeh W, Qamar R, Budway R, Levine E, et al. Association of 70-Gene Signature Assay Findings With Physicians' Treatment Guidance for Patients With Early Breast Cancer Classified as Intermediate Risk by the 21-Gene Assay. *JAMA Oncol*. 2018 Jan; 4(1):e173470.
- 68 Mitterperger L, Delahaye LJ, Witteveen AT, Spangler JB, Hassenmahomed F, Mee S, et al. MammaPrint and BluePrint Molecular Diagnostics Using Targeted RNA Next-Generation Sequencing Technology. *J Mol Diagn*. 2019 Sep;21(5):808–23.
- 69 Slembrouck L, Darrigues L, Laurent C, Mitterperger L, Delahaye LJ, Vanden Bempt I, et al. Decentralization of Next-Generation RNA Sequencing-Based MammaPrint® and BluePrint® Kit at University Hospitals Leuven and Curie Institute Paris. *Transl Oncol*. 2019 Dec;12(12):1557–65.
- 70 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 Mar;14(1):177.
- 71 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 Oct; 12(1):456.
- 72 Schildgen V, Warm M, Brockmann M, Schildgen O. Oncotype DX breast cancer recurrence score resists inter-assay reproducibility with RT2-profiler multiplex RT-PCR. *Sci Rep*. 2019 Dec;9(1):20266.
- 73 Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, et al.; Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet*. 2012 Feb;379(9814):432–44.
- 74 Thangarajah F, Eichler C, Fromme J, Malter W, Caroline Radosa J, Ludwig S, et al. The impact of EndoPredict® on decision making with increasing oncological work experience: can overtreatment be avoided? *Arch Gynecol Obstet*. 2019 May;299(5):1437–42.
- 75 Eichler C, Fromme J, Thangarajah F, Puppe J, Paepke S, Warm M, et al. Gene-expression Profiling - A Decision Impact Analysis: Decision Dependency on Oncotype DX® as a Function of Oncological Work Experience in 117 Cases. *Anticancer Res*. 2019 Jan;39(1):297–303.
- 76 Katz G, Romano O, Foa C, Vataire AL, Chantelard JV, Hervé R, et al. Economic Impact of Gene Expression Profiling in Patients with Early-Stage Breast Cancer in France. *PLoS One*. 2015 Jun;10(6):e0128880.
- 77 Lux MP, Nabieva N, Hildebrandt T, Rebscher H, Kümmel S, Blohmer JU, et al. Budget impact analysis of gene expression tests to aid therapy decisions for breast cancer patients in Germany. *Breast*. 2018 Feb;37:89–98.
- 78 Cejalvo JM, Martínez de Dueñas E, Galván P, García-Recio S, Burgués Gasión O, Paré L, et al. Intrinsic Subtypes and Gene Expression Profiles in Primary and Metastatic Breast Cancer. *Cancer Res*. 2017 May;77(9):2213–21.
- 79 Fabi A, Di Benedetto A, Metro G, Perracchio L, Nisticò C, Di Filippo F, et al. HER2 protein and gene variation between primary and metastatic breast cancer: significance and impact on patient care. *Clin Cancer Res*. 2011 Apr; 17(7):2055–64.
- 80 de Dueñas EM, Hernández AL, Zotano AG, Carrión RM, López-Muñoz JJ, Novoa SA, et al. Prospective evaluation of the conversion rate in the receptor status between primary breast cancer and metastasis: results from the GEICAM 2009-03 ConvertHER study. *Breast Cancer Res Treat*. 2014 Feb;143(3):507–15.
- 81 Falato C, Tobin NP, Lorent J, Lindström LS, Bergh J, Foukakis T. Intrinsic subtypes and genomic signatures of primary breast cancer and prognosis after systemic relapse. *Mol Oncol*. 2016 Apr;10(4):517–25.
- 82 Santa-Maria CA, Kruse M, Raska P, Weiss M, Swoboda A, Mutonga MB, et al. Impact of tissue-based genomic profiling on clinical decision making in the management of patients with metastatic breast cancer at academic centers. *Breast Cancer Res Treat*. 2017 Nov; 166(1):179–84.
- 83 Nixon MJ, Formisano L, Mayer IA, Estrada MV, González-Ericsson PI, Isakoff SJ, et al. PIK3CA and MAP3K1 alterations imply luminal A status and are associated with clinical benefit from pan-PI3K inhibitor buparlisib and letrozole in ER+ metastatic breast cancer. *NPJ Breast Cancer*. 2019 Sep;5(1):31.

- 84 André F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for PIK-3CA-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med*. 2019; 380(20):1929–40.
- 85 Robinson DR, Wu YM, Vats P, Su F, Lonigro RJ, Cao X, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet*. 2013 Dec;45(12):1446–51.
- 86 De Santo I, McCartney A, Migliaccio I, Di Leo A, Malorni L. The Emerging Role of ESR1 Mutations in Luminal Breast Cancer as a Prognostic and Predictive Biomarker of Response to Endocrine Therapy. *Cancers (Basel)*. 2019 Nov;11(12):E1894.
- 87 Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. *Nat Rev Cancer*. 2004 Oct;4(10):814–9.
- 88 Sønderstrup IM, Jensen MR, Ejlersten B, Eriksen JO, Gerdes AM, Kruse TA, et al. Subtypes in BRCA-mutated breast cancer. *Hum Pathol*. 2019 Feb;84:192–201.
- 89 Wu Y, Zhang Y, Pi H, Sheng Y. Current therapeutic progress of CDK4/6 inhibitors in breast cancer. *Cancer Manag Res*. 2020 May; 12:3477–87.
- 90 Militello AM, Zielli T, Boggiani D, Michiara M, Naldi N, Bortesi B, et al. Mechanism of action and clinical efficacy of CDK4/6 inhibitors in BRCA-mutated, estrogen receptor-positive breast cancers: case report and literature review. *Front Oncol*. 2019 Aug;9:759.
- 91 Litton JK, Rugo HS, Ettl J, Hurvitz SA, Gonçalves A, Lee KH, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. *N Engl J Med*. 2018 Aug; 379(8):753–63.
- 92 Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *N Engl J Med*. 2017 Aug; 377(6):523–33.
- 93 Kono M, Fujii T, Lim B, Karuturi MS, Tripathy D, Ueno NT. Androgen Receptor Function and Androgen Receptor-Targeted Therapies in Breast Cancer: A Review. *JAMA Oncol*. 2017 Sep;3(9):1266–73.
- 94 Drago JZ, Chandarlapaty S, Jhaveri K. Targeting Apoptosis: A New Paradigm for the Treatment of Estrogen Receptor-Positive Breast Cancer. *Cancer Discov*. 2019 Mar;9(3):323–5.
- 95 National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer, version 1. 2019 [cited 2020 May 22]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf.
- 96 AWOgyn Gynäkologische Onkologie e.V. (AGO). 2020 [cited 2020 May 22]. Available from: www.ago-online.de/fileadmin/ago-online/downloads/_leitlinien/kommission_mamma/2020/PDF_DE/2020D%205_Prognostische%20und%20praediktive%20Faktoren.pdf.