Prognostic and Predictive Role of Genetic Signatures

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12.1 Gene Expression Reveals Inter-tumor Heterogeneity: BC Intrinsic Subtypes

A robust body of evidence, initiated by the seminal studies of Dr. Perou's group at the dawn of the new millennium [1, 2] and repeatedly confirmed over the following decade, has convincingly demonstrated that breast cancer (BC) is a heterogeneous disease further classifiable in at least four molecular intrinsic subtypes (luminal A and B, HER2 enriched, basal-like, and normal breast), based on hierarchical clustering of the "intrinsic genes" (i.e., genes with minimal variation within a tumor sample, but maximal variation between different patients) expression profile. These studies were originally based on genome-wide gene expression profiling from microarray datasets and progressed to a PCR-based test with a list of 50 genes (the PAM50 gene signature) [3, 4] (Fig. 12.1).

Recently, the NanoString nCounter^{DX} Analysis System has been shown to provide more precise and accurate measures of mRNA expression levels in formalin-fixed, paraffin-embedded (FFPE) tissue when compared to PCR [5]. Actually, PCR-based assays require excessive optimization from archival FFPE samples, thus introducing amplification biases, due to high mRNA fragmentation and cross-links to protein upon fixation. Luminal A and B subtypes are largely distinguished by the expression of two main biological processes: proliferation-/cell cycle-related and luminal/hormone-regulated pathways. Compared to luminal A, luminal B tumors are characterized by higher expression of proliferation-/cell cycle-related genes or proteins (e.g., Ki-67) and lower expression of several luminal-related genes or proteins such as the progesterone receptor (PgR) and FOXA1, while estrogen receptor (ER) is expressed at similar levels in the two subtypes. ICH-classified early BC patients usually receive adjuvant systemic treatment in addition to local treatment (surgery and radiation therapy) depending on their clinicopathological subtype. Current guidelines indicate that all patients with ER-positive disease should receive at least 5 years of adjuvant endocrine therapy (ET) [6–8]. One of the milestones of the current classification is the distinction between luminal A-like and luminal B-like tumors with significant different clinical outcomes, resulting in different indication of adjuvant cytotoxic therapy [8]. The indication for chemotherapy (CHT) in patients with luminal disease has traditionally been based on the prognostic factors of tumor size, histological grade, Ki-67 levels, ER and PgR expression, and number of involved lymph nodes. Nevertheless, studies suggest a high variability on criteria used to add CHT in the setting of luminal disease [9]. Previous studies showed that more than 60% of hormone receptor-positive BC patients receive adjuvant cytotoxic therapy. However, other authors provided evidence that only 4–5% of these women would likely benefit of this therapy [10]. Considering that serious and even life-threatening toxicities (bleeding, neutropenic fever, transfusion requirement, congestive heart failure, secondary malignancy, and peripheral neuropathy) occur in approximately 1–2% of patients, it is evident that conceiving a more specific prognostic system is mandatory in order to identify patients who can avoid CHT. The St. Gallen International Expert Consensus Panel adopted an intrinsic subtype-based approach for recommending adjuvant systemic therapies (i.e., ET, CHT, and anti-HER2 therapy) in early BC [8]. Although acknowledging the higher accuracy and reproducibility of gene expression assays, the panel recognized that they are not easily available for all BC patients, due to technical and especially economical reasons. As a result, immunohistochemistry (IHC)-based methods with antibodies recognizing ER and PgR, HER2, and Ki-67 are currently used as a surrogate for intrinsic subtypes, as detailed in Fig. 12.2.

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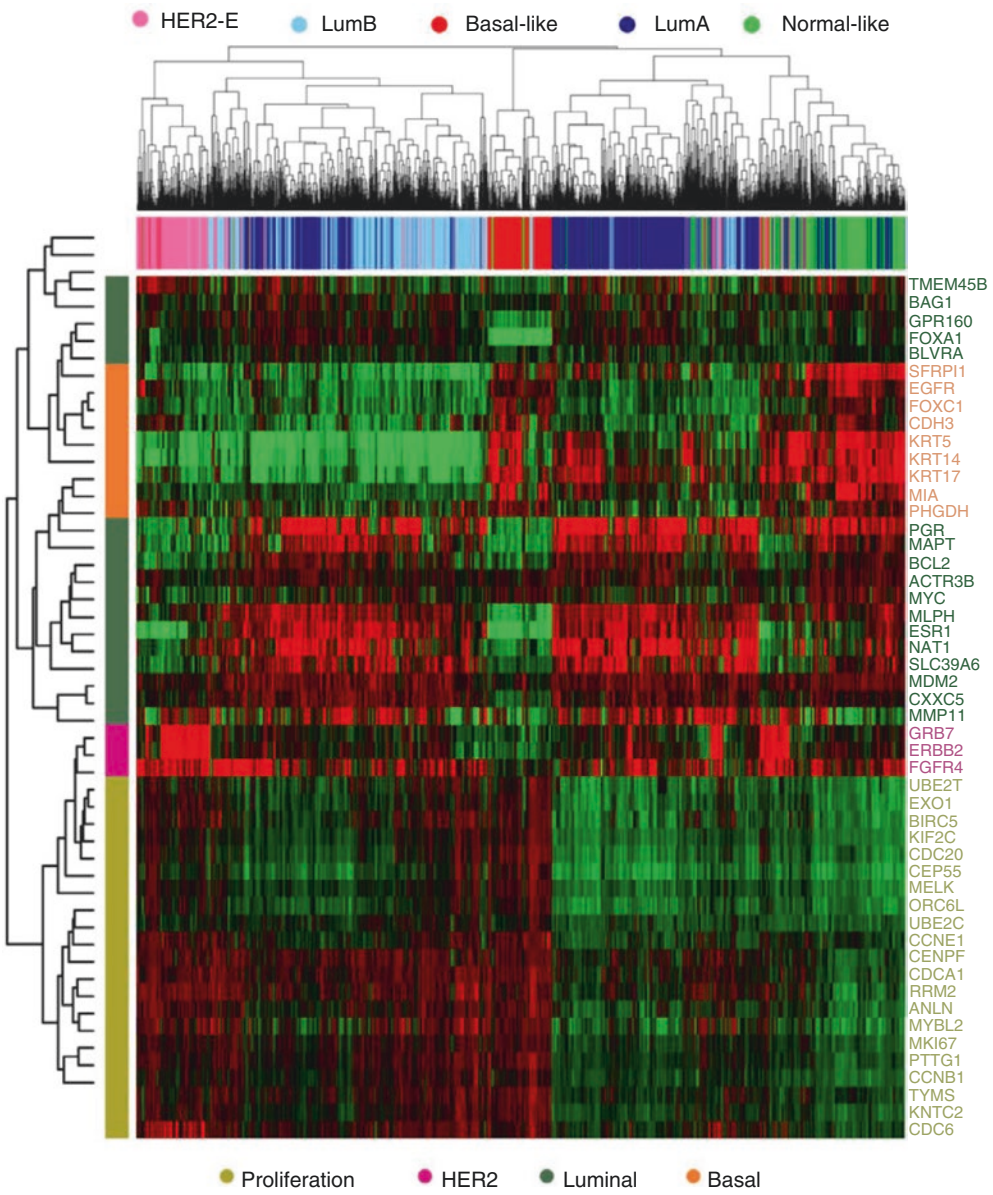

Fig. 12.1 Intrinsic subtype identification using the PAM50 classifier. The subtype calls of each sample are shown below the array tree. The expression values are shown as *red*/*green* according to their relative expression level [4]

Although the identification of intrinsic subtypes by IHC is widely distributed and relatively inexpensive, it is limited by interobserver variability and technical reproducibility [11]. Furthermore, several studies proved that IHC is not completely reproducible in identifying intrinsic subtypes, possibly due to the fact that four antigens do not fully recapitulate an intrinsic subtype originally identified by the expression of 50 genes: across the IHC-identified subtypes, the discordance rate is 38% for luminal A and 49% for luminal B [4]. Studies in the neoadjuvant setting provided indirect evidence that luminal A are less sensitive to CHT than luminal B tumors, thus achieving a significant lower rate of pathological complete response (pCR) when treated by different CHT schemes [4]. This is further sustained by the fact that pCR is prognostic in luminal B, but not in luminal A BC patients. The 2015 St. Gallen International Expert Consensus [12] recognized that luminal A-like BC are less responsive to CHT and should be therefore treated with ET only, with the exception of cases with extensive (four or more lymph nodes) axillary involvement. Oppositely, CHT in combination with ET is usually recommended for luminal B-like BC patients, unless they are bearing clinicopathological low-risk features, including T1 size, no or limited (1–3 nodes, pN1a) nodal involvement, absence of peritumoral vascular invasion, and very high ER/PgR and/or low Ki-67 values. Collectively, these data provided the rationale

for a better identification of which population of ER-positive (luminal) BC has a risk of relapse low enough to allow sparing a noneffective and potentially harming CHT treatment.

Intrinsic subtype	Clinico-pathologic surrogate definition
Luminal A	**'Luminal A-like'** *all of:* ER and PgR positive HER2 negative Ki-67 'low' Recurrence risk 'low' based on multi-gene-expression assay (if available)
Luminal B	**'Luminal B-like (HER2 negative)'** ER positive HER2 negative and *at least one of:* Ki-67 'high' PgR 'negative or low' Recurrence risk 'high' based on multi-gene-expression assay (if available) **'Luminal B-like (HER2 positive)'** ER positive HER2 over-expressed or amplified Any Ki-67 Any PgR
Erb-B2 overexpression	**'HER2 positive (non-luminal)'** HER2 over-expressed or amplified ER and PgR absent
'Basal-like'	**'Triple negative (ductal)'** ER and PgR absent HER2 negative

Fig. 12.2 Immunohistochemical surrogate definition of luminal intrinsic subtypes [8]

12.2 Multiparametric Molecular Markers: The Candidates

Using different techniques to measure mRNA levels, including RT-PCR and DNA microarrays, the assays shown in Fig. 12.3 have been basically designed to measure the risk of BC recurrence.

12.3 MammaPrint® 70-Gene Recurrence BC Assay

MammaPrint® is one of the first gene expression arrays approved by FDA for commercial use. It measures the mRNA expression of 70 genes, focusing primarily on proliferation, with additional genes associated with angiogenesis, metastasis, invasion, and stromal integrity [13–16]. This test was developed without an a priori knowledge of the role of the involved genes using a data-driven approach. Initially, about 5000 genes were found to be significantly deregulated across 78 BC patients treated at the Netherlands Cancer Institute [13]. All the patients were node negative, with tumor measuring less than 5 cm in diameter, were aged <55 years, and were selected irrespectively of their hormonal receptor status. Using a supervised classification method by correlating the expression of each gene with the disease outcome, the authors ended up with a core of 70 candidates bearing significant prognostic value [13]. The expression profile of these genes allowed to identify two patient subgroups, with "good prognosis" or "poor prognosis," having appreciable different risks to develop distant metastasis within 5 years. When the patients were classified according to the St. Gallen and National Institute of Health (NIH) criteria, the 70-genes score was found to be able to reduce the risk of overtreatment by

	21-gene RS (Oncotype Dx®)	Amsterdam 70-gene signature (Mammaprint®)	PAM50 (Prosigna™)	Rotterdam 76-gene signature	Genomic grade index	Breast cancer index	Endopredict®
Relevant EBC Population	ER+ HER2- Node-	Node- Tumor size ≤5 cm	ER+	Node-	ER+	ER+ Node-	ER+ HER2-
Tissue Required for Assay	FFPE	FFPE or frozen	FFPE	FFPE	FFPE or frozen	FFPE	FFPE
Assay Technique	qRT-PCR	Microarray	qRT-PCR	Microarray	Microarray	qRT-PCR	qRT-PCR
Demonstrated Analytic Validity	✓	✓	✓				✓
Demonstrated Clinical Validity	✓	✓	✓	✓	✓	✓	✓
Demonstrated Clinical Utility	✓		✓			✓	✓
Level of Evidence	IB	III	IB	III	III	IB	IB
Ongoing Studies	TAILORx, RxPONDER	MINDACT					

Fig. 12.3 Multigene prognostic tests for BC patients [Cobain EF, Hayes DF Curr Treat Options Oncol 2015]

25–30%. In particular, MammaPrint®, St. Gallen, and NIH systems assigned 40, 15, and 7% of the patients to the low-risk category, respectively. In a multivariable analysis, MammaPrint® showed a stronger independent prognostic ability than the matched clinicopathologic factors [17, 18]. The assay was then tested in a larger series of 295 patients including either ER-positive or ER-negative BC from the same institution, confirming its prognostic ability in predicting 10-year survival outcome. In particular, within the lymph node-negative sub-cohort (151 patients), the 10-year distant disease-free survival was 87% for the low-risk group and 44% for the high-risk group [19]. These data provided evidence that MammaPrint® was an high performing test in prognosticating ER-positive BC patients, outdoing current clinicopathological characteristics, while its clinical validity was much lower in the ER-negative setting, where nearly all of the patients were classified as high risk. Along this line, a number of retrospective analyses confirmed that only ER-positive patients within the high-risk category did benefit from adjuvant CHT [19–23] suggesting that MammaPrint® could also be used as a predictive tool. The first prospective study (RASTER), conducted in 16 community hospitals in the Netherlands, confirmed the feasibility of the 70-genes test [23] and evaluated its overall performance rate. The study enrolled 427 patients younger than 61 years with T1–T3, node-negative BC, irrespective to their hormonal receptor status. Patients received the adjuvant systemic treatment recommended by the 2004 Dutch Institute of Healthcare Improvement guidelines [24], also taking into account physicians' and patients' preferences. The results of the 70-genes classification were compared with Adjuvant! Online (AOL) [25]. This is a web-based tool for estimating risk of relapse and mortality and illustrating the benefits provided by various treatment regimens for newly diagnosed BC patients. The estimates for risk of death are derived from the Surveillance, Epidemiology, and End Results (SEER) data. AOL!® estimates recurrence by adding 14% to the mortality risk to account for the risk of contralateral breast cancer and local/regional events unlikely to result in breast cancer mortality. The estimates of treatment benefit are derived from available clinical trial results and data from the 1995 Overview meta-analyses of randomized adjuvant chemotherapy and hormone therapy trials for breast cancer [26], with supplemental information from the 2000 Overview [27]. AOL!® and MammaPrint® yielded to a 38% discordance rate in risk estimations, with most of the discordant cases being low risk according to the 70-gene signature and high risk according to AOL!®. The majority (98%) of these patients who did not receive adjuvant CHT showed an uneventful clinical course. Based on these data, it has been concluded that patients pertaining to the MammaPrint® low-risk group would spare CHT without any negative impact on recurrence rate [22]. The first independent validation study, performed by the TRANSBIG research consortium, used samples from 302 patients younger than 60 years and with node-negative, T1–T2 BC. This study confirmed the ability of MammaPrint® in discriminating patients' outcome with Hazard Ratio of 2.79 (95%CI, 1.60-4.87) and 2.32 (95%CI, 1.35-4.0) respectively, for distant metastasis and overall survival, outperforming clinicopathological characteristics [28]. According to the evidence that up to 25–30% of node-positive BC patients would remain free of distant metastasis even without any adjuvant CHT [29], a retrospective study selected 241 T1, T2, or operable T3, pN1a (1–3 metastatic lymph nodes) BC patients [20]. In this cohort, good-prognosis patients (41%) showed a 91% 10-year distant metastasis-free survival and a 96% BC-specific survival, respectively, while both the survival rates were 76% in the poor-prognosis group. Multivariable analysis showed that the 70-gene signature was the most powerful independent predictor for BC-specific survival, with a HR of 7.17, confirming its utility in identifying patients who can safely spare adjuvant CHT even if node positive [20]. Straver et al. [16] assessed the role of 70-gene assay in the neoadjuvant setting in a cohort of 171 patients with BC larger than 3 cm and/or with positive lymph nodes at diagnosis, finding that, as expected, 86% of the patients showed the high-risk signature. The rate of pCR was 20% for high-risk and 0% for low-risk patients [16]. In February 2007, the TRANSBIG consortium launched a multicenter, prospective, and randomized controlled study, the "microarray for node-negative disease may avoid CHT" trial, whose results have been eventually presented in 2016 at the AACR meeting and published soon thereafter [30, 31]. Out of the 11,288 patients enrolled, the trial assessed the risk in 6693 early BC patients by either AOL or the 70-gene assay. The 2142 (31.2%) patients with discordant results have been randomized to receive the adjuvant treatment dictated by AOL or gene expression profile, i.e., ET only for low-risk patients and ET + CHT for high-risk patients [30, 31]. The remaining patients, placed into the same risk category by both methods, were treated in accordance with the current guidelines (ET for the low-risk group and ET + CHT for the high-risk group). A total of 1550 patients (23.2%) were classified as high clinical risk and low genomic risk. At 5 years, the rate of survival without distant metastasis in this group was 94.7% (95% CI, 92.5–96.2) among those not receiving CHT. The absolute difference in survival rate between these patients and those who received CHT was 1.5% points [30, 31].

12.4 Oncotype DX® (Genomic Health 21-Gene Recurrence Score)

This assay evaluates the RNA expression of a panel of 21 genes (16 cancer-related genes and five reference genes) by RT-PCR, providing information about the 10-year risk of distant recurrence (DR). The 21-genes panel works in FFPE samples and includes genes involved in tumor cell proliferation (representing five of the 16 cancer-related genes),

invasion, HER2, and hormone response. The relative expression levels of these genes is calculated by a mathematical algorithm mostly weighting proliferation genes that generate the Recurrence Score (RS), expressed as a value between 0 and 100. RS provides a quantitative risk of distant recurrence and stratifies patients in three categories: low risk (RS < 18), intermediate risk (RS 18–30), and high risk (RS > 30) [32]. RS has been validated as an independent prognostic measure of the risk of recurrence for women with ER-positive, lymph node-negative early BC treated by ET only, outperforming traditional clinicopathological characteristics of patient age, tumor size, and grade [32, 33]. In 2010, a retrospective study examined specimen collected within the ATAC trial with the objective to evaluate the prognostic value of the Oncotype in the postmenopausal setting [34]. The ATAC trial evaluated the efficacy and safety of 5 years of anastrozole, tamoxifen, or the combination of both in postmenopausal women and selected more than 5000 women with localized invasive BC and ER-positive disease [35]. 76% of the specimens from this collection was then used to confirm the performance of RS in elderly patients, demonstrating that RS was an independent predictor of recurrence in both nodes-negative and nodes-positive patients [34]. Moreover, the RS was found to be a strong predictive factor of benefit from cyclophosphamide, doxorubicin, and fluorouracil and fluorouracil (CAF) in ER-positive, node-positive, postmenopausal BC patients. Patients classified as low risk did not derive any benefit from CHT, while a significant advantage from treatment with CAF was observed in patients with a high RS [36]. Likewise, in a cohort of 89 patients with locally advanced BC treated preoperatively with paclitaxel and doxorubicin, the probability of pCR was shown to increase along with RS [37]. Oncotype DX® RS is widely used in the USA, allowing to spare CHT in approximately one third of the cases [38–40] and resulting in overall cost reduction for the health system [41]. A prospective clinical study, the Trial Assigning IndividuaLized Options for Treatment (Rx) (TAILORx), is currently seeking to incorporate the Oncotype DX® test into clinical decision-making, in order to spare women unnecessary treatment if CHT is not likely to be of substantial benefit [42]. TAILORx recruited more than 10,000 women with node-negative, ER- and/or PgR-positive, HER2-negative invasive BC. For this trial, the original RS threshold values have been modified as follows: below 11 (vs. <18) for the low-risk group, from 11 to 25 (vs. 18–30) for the intermediate-risk group, and above 25 (vs. ≥31) for the high-risk group. Low- and high-risk patients have been assigned to ET only or ET + CHT, respectively, while patients with an intermediate (11–25) RS have been randomly assigned to receive ET + CHT or ET only. Sparano et al. [43] recently reported an interim analysis of the TAILORx trial. Patients had tumors measuring 1.1–5.0 cm in the greatest dimension (or 0.6–1.0 cm and G2/G3 tumor grade) and met established guidelines for the consideration of adjuvant CHT on the basis of clinicopathological features. A total of 1626 patients were assigned to receive ET without CHT if they had a recurrence score of 0–10, indicating a very low risk of recurrence. The 5-year rate of invasive DFS was 93.8% (95% CI 92.4–94.9), the rate of freedom from recurrence of BC at a distant site was 99.3% (95% CI, 98.7–99.6), and the rate of OS was 98.0% (95% CI, 97.1–98.6). These data supported the application of 21-genes assay to select patients who may be safely spared CHT treatment [43]. Several studies argued that RS does not provide prognostic information beyond traditional clinicopathological characteristics (i.e., ER/PR receptor, Ki-67 labeling index, and tumor grade), criticizing the cost-effectiveness of the Oncotype DX test [44–52]. In particular, Gage et al. [53] recently interrogated a population of 540 BC patients, reporting that 55% of the study population that would have met the criteria for Oncotype DX® testing (node-negative, ER-positive, HER2-negative invasive BC) would be easily classified in the low- and high-risk category by traditional tools, thus not needing additional information. Specifically, patients with high tumor grade or low (<20%) ER immunoreactivity should be considered at high risk of distant relapse, while patients with low tumor grade and highly ER and PgR express tumors at low risk. The authors concluded that only patients bearing intermediate features would benefit of the Oncotype DX® testing, leading to significant cost savings [53]. Taking into account that RS increases in relation to the levels of proliferation genes and that patients with higher RS benefit from adjuvant CHT, Baxter et al. [54] investigated the prognostic relevance of traditional biomarkers of proliferation (mitotic count and Ki-67 labeling index) in 226 ER-positive, HER2-negative, T1/T2, node-negative BC patients referred to British Columbia Cancer Agency in 2007–2011. The authors found that tumors with a low/intermediate Nottingham grading or low mitotic count were unlikely to be classified as high risk by Oncotype DX®, suggesting to test only patients with high histological score [54].

12.5 EndoPredict®

The EndoPredict® (EP) assay was developed for early, ER-positive, HER2-negative BCs, in order to identify patients with a low rate of recurrence without adjuvant cytotoxic therapy. Based on a RT-PCR method, EP evaluates the expression levels of eight cancer genes (AZGP1, BIRC5, DHCR7, IL6ST, MGP, RBBP8, STC2, UBE2C) and three control genes (CALM2, OAZ1, RPL37A) in FFPE tissue. These genes are related to tumor proliferation and to hormone receptor activity, but do not include ESR1, PgR, or HER2, at variance with Oncotype DX® and PAM50 assays. EP classifies patients treated with adjuvant ET only into a low- or a high-risk category, and it is feasible in a decentralized setting [55–57]. The EP score ranges between 0 and 15 with a threshold of 5 to discriminate between the low- and

high-risk categories. EP was developed and validated in over 1000 postmenopausal, node-negative and node-positive ER-positive and HER2-negative BC samples, retrospectively collected in prospective clinical trials [58]. Continuous EP score proved its prognostic role for distant recurrence, outperforming the established clinicopathological variables of ER immunoreactivity, Ki-67 labeling index, and AOL. The EP score has been subsequently integrated by the clinical characteristics of nodal status and tumor size to create a linear model risk score called EPclin that in turn proved to be a powerful prognostic marker, resulting in a 10-year recurrence rate of 4% for the EPclin low-risk group and 22–28% for the high-risk group [59]. EPclin identifies a subgroup of patients with an excellent long-term prognosis after 5 years of ET, confirming its prognostic ability for both early and late relapse and suggesting that the low-risk patient subgroup might not need an extended ET [58]. The EPclin score has also been found to outperform purely clinical risk classifications (St. Gallen, German S3, and NCCN). Among 1702 ER-positive/HER2-negative, postmenopausal women treated with exclusive ET, 58–61% of patients classified as high/intermediate risk according to clinical guidelines were reassigned to the low-risk group by the EPclin score [60]. In a retrospective study dealing with 167 patients with ER-positive, HER2-negative BC, EPclin score led to a change of the planned therapy in 37.7% of patients, shifting to CHT in 12.3% and to exclusive ET in 25% [61]. The interaction between EP score and CHT has also been investigated: Bertucci et al. [62] collected 553 ER-positive and HER2-negative BC pretreatment core biopsies samples for which documentation of pathological response to anthracycline-based neoadjuvant CHT was available, finding that the high-risk group had a higher pCR rate than the low-risk group (17 vs. 7%). Martin et al. [63] reported a prospective-retrospective clinical validation trial designed to investigate whether EP can safely be used to identify node-positive BC patients who can avoid CHT. In this cohort of 1246 ER-positive, HER2-negative, node-positive, CHT (5-fluorouracil, epirubicin, and cyclophosphamide with or without 8 weekly courses of paclitaxel)-treated BC patients, 25% were classified as low risk on the basis of the EP score. In this subgroup, 93% of the patients showed a distant metastasis-free survival, compared to 70% in the high-risk group.

12.6 PAM50® and Risk of Recurrence (ROR) Score

This assay was developed to classify tumors according to the intrinsic subtype (see above) and to improve the classification concordance reported by investigators and is based on the relative expression of 50 genes [3]. Provided that the data are normalized, the test is considered a robust assay with a high concordance between laboratories [64]. The PAM50 classifier was validated in a cohort of 348 patients receiving tamoxifen, where it was found to outperform IHC in providing prognostic information and in predicting tamoxifen efficacy [65]. In a population of 151 ER early-stage BC patients, PAM50 achieved a good level of agreement with Oncotype DX® in identifying both high (luminal B and RS > 31)- and low-risk groups (luminal A and RS < 18) [66]. Within the group of Oncotype DX® intermediate RS, PAM50 classified 59% of the patients as luminal A, 33% as luminal B, and 8% as HER2 enriched. Moreover, Ki-67 labeling index was found to be reliable in distinguishing luminal A from luminal B and low-risk from high-risk RS tumors but not between the intermediate- and low-risk RS categories [66]. Adopting an algorithm that incorporates gene expression data, intrinsic subtype, and tumor size, Parker et al. [3] created the risk of recurrence (ROR) score (Prosigna), which stratifies patients in high, medium, and low subsets. The clinical utility of PAM50 and ROR score as a prognostic tool has been repeatedly reported in the ER-positive, HER2-negative setting [65, 67, 68].

12.7 Rotterdam 76-Gene Signature

This assay was developed at the Erasmus University Cancer Center in Rotterdam and made commercially available in 2005. The 76 genes included in this assay are mainly related to proliferation. The test was developed from the analysis of 115 women with node-negative BC (ER positive and ER negative), not receiving any adjuvant treatment and followed for more than 8 years, and differentiates patients in two categories, i.e., good signature or poor signature [69]. It has been reported to be highly predictive of distant relapse at 5 and 10 years. Desmedt et al. [70] found in a cohort of 198 node-negative untreated patients that the 5- and 10-year time to distant metastasis was 98 and 94% for the good profile group and 76 and 73% for the poor signature group, respectively. These data stemmed from retrospective analyses and still need to be confirmed in prospective randomized studies. The analytic validity, clinical utility, and reproducibility across different laboratories have not yet been confirmed.

12.8 Genomic Grade Index

Histologic grade is one of the best-established prognostic biomarkers in BC, providing reliable information regarding tumor behavior [71, 72]. However, the Elston-Ellis histological grading system shows low reproducibility among pathologists [73] and does not provide clear prognostic information for patient with grade 2, which represents the majority of cases [74]. The genomic grade index (GGI) was developed

with the aim of grading breast tumors more accurately than the conventional histological grade in the ER-positive, HER2-negative setting [75]. It was developed in 189 BC patients and validated in an independent cohort of 597 cases. The authors created a two-tier classification system based on the differential expression of 97 genes mainly involved in cell cycle regulation and proliferation. The level of expression of these genes was found to reclassify grade 2 tumors into high and low genomic grade category. High GGI score patients were associated with a higher risk of recurrence than low GGI score patients (HR = 3.61, 95% CI = 2.25–5.78) [76]. Loi et al. [77] demonstrated the prognostic ability of GGI in stratifying luminal tumors, reporting that luminal A and B tumors fall into the GGI low risk and high risk, respectively. The role of GGI in predicting pathological response to neoadjuvant CHT was investigated in 229 fine-needle biopsies of BC patients treated with a taxane- and anthracycline-containing neoadjuvant therapy. In this study, a high GGI score was an independent predictor of response to CHT [78].

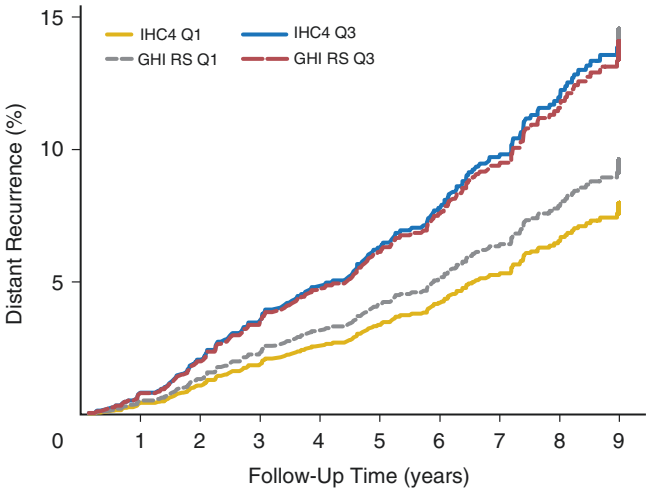

Fig. 12.4 Example of predicted time to distant recurrence for a node-negative postmenopausal patient with a G3 1–2 cm tumor treated with anastrozole who is at either the 25th (quartile 1 [Q1]) or 75th (Q3) percentile of the IHC4 score (score for four immunohistochemical markers: estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, and Ki-67) or the Oncotype DX® Genomic Health recurrence score (GHI-RS) [82]

12.9 BC Index (BCI)

It is a RT-PCR-based assay working in FFPE samples, based on the HOXB13-to-IL17BR expression ratio (H:I ratio) and the molecular grade index (a five-gene molecular grade index, primarily consisting of proliferation-related genes) [79]. This assay was developed using a cohort of ER-positive tamoxifen-treated BC patients and has been shown to provide an individual risk of distant BC recurrence based on a continuous risk model [80]. The main strength of the BCI is its capability of predicting the risk of both early (within 5 years) and late (10 years) recurrences in ER-positive, node-negative BC. Indeed, the assay was retrospectively evaluated in two cohorts of 317 and 358, ER-positive, node-negative tamoxifen-treated patients. In both cohorts, continuous BCI was found the most significant prognostic factor beyond standard clinicopathologic factors, both for early and late events [81].

12.10 Comparative Evaluation of Prognostic Performance of Multigene Tests and Clinicopathological Characteristics

Most of the comparative data on multigene prognosticators have been obtained by Dr. Dowsett lab taking advantage of the samples prospectively collected within the TransATAC, the translational substudy of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial [35]. The ATAC trial randomized ER-positive, HER2-negative BC patients to receive exclusively tamoxifen or anastrozole for 5 years, with distant relapse as the primary end point. Cuzick et al. [82] comparatively analyzed the performance of Oncotype DX RS and IHC4 score (resulting from the immunohistochemical evaluation of ER, PgR, HER2, and Ki-67 integrated into the IHC4 score). The authors found that the information provided by the IHC4 score and Oncotype DX® were similar and that little additional prognostic value was seen combining both scores (Fig. 12.4).

Dowsett et al. [68] then compared the prognostic relevance of PAM50 ROR score with Oncotype DX RS and IHC4 in 940 ER-positive BC patients. The ROR score added significant prognostic information for distant relapse in the whole population ($p < 0.001$) and in HER2-negative/node-negative patients. Likewise, PAM50 ROR provided additional prognostic information beyond Oncotype DX® RS in the overall population and within each subgroup. Interestingly, relatively similar information was provided by ROR and IHC4 in all patients. Buus et al. [83] recently compared the prognostic information provided by Oncotype DX®, EP/EPclin, and clinical treatment score (CTS, obtained by integrating the prognostic information from nodal status, tumor size, histopathological grade, age, and anastrozole or tamoxifen treatment) in 928 ER-positive, HER2-negative BC patients enrolled in the anastrozole and tamoxifen arms of the ATAC trial, with distant relapse-free survival as the primary end point. In the overall population, EP and EPclin provided substantially more prognostic information than Oncotype DX RS, especially with regard to the risk of late relapse and in node-positive patients. In a prospective comparison study, conducted on 665 patients with ER-positive, node-negative BC patients, BCI was com-

pared with the Oncotype DX® 21-genes recurrence score. Both the assays demonstrated significant prognostic ability for early distant recurrence, while only BCI was significant for late distant recurrence [84].

12.11 Concordance Among Gene Expression Tests

It has been demonstrated that gene expression-based tests show only moderate concordance. For example, Oncotype Dx® and EndoPredict® had a 24% discordance in a study reported by Dowsett et al. [83]. This level of concordance is similar to that reported between Oncotype Dx- and Ki67-based risk algorithms. The fact that even gene expression-based tests show a nonnegligible discordance rate when ran in a single patient, coupled with the reality of medical practice in countries where gene expression-based tests are not affordable owing to high cost (in Italy, the out-of-pocket cost for patients is >$4000), underlines the importance to limit the use of gene expression tests to subgroups of ER-positive BC patients and to put efforts to validate Ki-67 (as a surrogate of proliferation) as a predictive marker for CHT benefit.

12.12 Toward a Rationale Use of the Multigene BC Prognosticators

The aforementioned gene expression-based prognostic tests are highly reliable in informing on ER-positive/node-negative BC patients prognosis, and most of them achieved a level of evidence 1B, being validated in samples retrospectively collected within prospective randomized clinical trial. Furthermore, MammaPrint® and Oncotype DX® have been recently validated in prospective clinical trials using molecular data as a randomization factor. This notwithstanding, the multigene tests are not widely used in the daily practice, especially for their high costs and the need of sample centralization. A meta-analysis of BC microarray gene expression profiling data [85] demonstrated that they are all basically looking at the same process: tumor cell proliferation. In other words, they essentially discriminate ER-positive BCs with low proliferation (luminal A intrinsic subtype) and low clinical risk, from ER-positive BCs with high proliferation (luminal B intrinsic subtype) and high clinical risk [85–87]. Multigene tests are used for assessing whether a patient with an early ER-positive BC should receive CHT, and in this regard they may be considered as a potential biomarker. Although their analytical and clinical validity has been convincingly demonstrated, uncertainties remain concerning their clinical utility. A biomarker has clinical utility if its application is associated with a significant survival benefit: it should outperform preexisting clinicopathological indicators or, alternatively, provide comparable information at lower cost, less invasively, or with less morbidity. Nevertheless, these advantages are still not enough for achieving clinical validity: as stated by the recently issued ASCO guidelines for the use of biomarkers in early BC [88], "the magnitude of the benefit must be clinically meaningful and outweigh risks, costs, and/or inconvenience associated with use of the test and the degree of benefit required to recommend for or against a treatment must be tempered with clinical judgment and patient perspective." For example, giving adjuvant CHT to triple negative, node-positive BC patients is of clinical utility beyond any doubt: the 10-year likelihood of incurable distant recurrence in this setting would exceed 50% in the absence of adjuvant CHT that indeed reduces the risk of recurrence by 30%, with a 15–20% absolute benefit and an odd of fatal, life-threatening, or permanent life-changing toxicities accounting for 2–3%. On the other hand, the 10-year risk of recurrence for luminal A-like BC patients (ER/PgR highly expressed, HER2 negative, <20% Ki-67, and/or G1) does not exceed 10% with ET only. This means that adding CHT, that would reduce the risk of recurrence by 30% in this setting as well, would yield to a 3% absolute benefit, which is roughly the same figure of patients potentially harmed. The 2015 St. Gallen International Expert Consensus [12] recognizes that luminal A-like BCs are less responsive to CHT and should be therefore treated with ET only, with the exception of cases with extensive (four or more lymph nodes) axillary involvement. Oppositely, CHT in combination with ET is usually recommended for luminal B-like BC patients, unless they are bearing clinicopathological low-risk features, including T1 size, no or limited (1–3 nodes, pN1a) nodal involvement, absence of peritumoral vascular invasion, and very high ER/PgR and/or low Ki-67 values, as well as multiparameter molecular markers of favorable prognosis [12]. The fact that these multigene tests have been validated only for node-negative patients (with the exception of MammaPrint® that has been used in 1–3 node-positive patients within the MINDACT trial) is anything but trivial in the clinical practice. ET is usually delivered to patients with luminal tumors characterized by favorable prognostic markers, including high levels of ER/PgR immunoreactivity, small T size (T1/T2), and absence of lymph node involvement, thus questioning the usefulness of running multigene tests in N0 luminal patients. Oppositely, patients with extensive lymph node involvement usually receive CHT irrespective of the biology of their tumor. As a consequence, multigene tests would be clinically useful specifically in patients with 1–3 positive lymph nodes. In this regard, Gnant et al. [89] recently investigated the prognostic role of PAM50 ROR in 543 patients with one to three node-positive BC treated with 5 years of adjuvant ET only within two phase III adjuvant trials: ABCSG-8 and ATAC. The authors found that the patients with one positive

lymph node classified as low risk by PAM50 ROR had a 6.6% risk of distant recurrence at 10 years (95% CI 3.3–12.8%). By contrast, low-risk patients with two or three positive lymph nodes nearly doubled the risk of distant recurrence to 12% (95% CI 6.6–22.8%). Assuming that CHT could reduce the risk of recurrence by 30%, these data prompt to speculate that ROR low-risk patients should receive CHT when 2–3 lymph nodes are involved, while the benefit of any further treatment in patients with just one metastatic lymph node would be negligible. Interestingly, patients without any lymph node involvement are more frequently classified as low risk by different multigene tests. Notwithstanding these data, the putative value of genomic tests in decision-making of luminal BC patients with 1–3 node-positive disease remains to be established. The MINDACT trial [30] reported that the 5-year rate of survival without distant metastasis in the clinical high-risk/molecular low-risk group was 94.7%, indipendently of the occurrence of lymph node metastasis. There are three further prospective phase III randomized trials (TAILORx and RxPONDER using Oncotype DX® and ASTER 70s using genomic grade) currently addressing the role of multigene tests in predicting adjuvant CHT benefit also in patients with up to three node-positive luminal BC, providing soon level 1 evidence on their clinical utility in daily practice. For the time being, ASCO guidelines recommend not to use multigene tests for ER-positive BC patients with lymph node involvement [88].

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