Intrinsic Subtypes of Primary Breast Cancer – Gene Expression Analysis

Marcus Schmidt, Christoph Thomssen, Michael Untch

Summary

Intrinsic subtypes based on gene expression have substantially improved the understanding of the biological diversity and heterogeneity of breast cancer. The efficacy of adjuvant therapies depends on the level of risk for an individual patient. Because of this, careful estimation of the level of risk is mandatory. In addition to well-established clinicopathological factors, validated gene expression signatures are useful especially in estrogen receptor-positive and HER2-negative patients. Commercially available gene expression signatures like Prosigna, MammaPrint, Oncotype DX, and EndoPredict are recommended by the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) for use in selected patients.

Introduction

It is self-evident that the magnitude of chemotherapy benefit depends on the level of risk of the individual patient [1]. In order to avoid over- as well as under-treatment it is advisable to select the appropriate treatment strategy on the basis of a careful risk assessment for each individual patient. According to the most recent St. Gallen consensus recommendations, conventional clinicopathological factors, validated gene expression signatures are useful especially in estrogen receptor-positive and HER2-negative patients. Precisely, it is self-evident that the magnitude of chemotherapy benefit depends on the level of risk of the individual patient [1]. In order to avoid over- as well as under-treatment it is advisable to select the appropriate treatment strategy on the basis of a careful risk assessment for each individual patient. According to the most recent St. Gallen consensus recommendations, conventional clinicopathological factors, validated gene expression signatures are useful especially in estrogen receptor-positive and HER2-negative patients. Commercially available gene expression signatures like Prosigna, MammaPrint, Oncotype DX, and EndoPredict are recommended by the Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) for use in selected patients.

Evolution of Tumor Markers in Breast Cancer

Of the multitude of new prognostic and predictive factors that have emerged in addition to traditional clinicopathological factors like tumor size, nodal status, histological grade, and lymphovascular invasion, only very few (estrogen receptor (ER), human epidermal growth factor receptor (HER2)) have gained a high level of evidence (LoE) and made their way into clinical practice. To improve the quality of research on biomarkers, a guideline named REMARK (Reporting Recommendations of Tumor Marker Prognostic Studies) was defined. REMARK describes the information that should be given when publishing a biomarker study such as study design, preplanned hypotheses, patient and specimen characteristics, assay methods, and statistical analysis methods [3]. Depending on the quality of a biomarker study, the Tumor Marker Utility Grading System assigns different LoE to new markers [4]. To obtain the highest LoE, a marker had to be tested prospectively in a prospective randomized clinical study. Recently, a refined system for biomarker study design and evaluation that incorporates a revised LoE scale for tumor marker studies, including those using archived specimens, was introduced (table 1) [5]. Although fully prospective randomized clinical trials to evaluate the medical utility of a prognostic or predictive biomarker are still considered the gold standard, such trials are costly, and more efficient indirect ‘prospective-retrospective’ designs using archived specimens might reach LoE I if validated with consistent results. The requirements to obtain LoE IB based on prospective-retrospective studies were recently described by McShane & Hayes [6]: i) adequate and representative amounts of archived specimens must be available from enough patients from a prospective trial; ii) analytical and preanalytical validation for use with archived specimens; iii) the plan for marker evaluation should be completely specified and should be focused on evaluation of a single completely defined marker-based test; and iv) the results from archived specimens should be validated in at least 1 similar study.
Gene expression profiling has allowed researchers to gain an insight into the heterogeneous nature of breast cancer. Perou et al. [10] described specific breast cancer subtypes identified after 2-dimensional hierarchical clustering, which they called luminal, basal-like, normal-like, and ERBB2-like. These intrinsic subtypes could be recapitulated in different gene expression datasets [13]. They differed in clinical outcome [14], preferential site of relapse [15], and response to chemotherapy [16], supporting the idea that many of these breast tumor subtypes represent biologically distinct disease entities. However, these early studies were hampered by the fact that fresh-frozen tissue was needed. To overcome this restraint and to develop a clinical test for intrinsic subtype diagnosis, Parker et al. [17] started with 1,906 ‘intrinsic’ genes that were analyzed by hierarchical clustering. In a next step, some of the breast cancers were also profiled by quantitative real-time polymerase chain reaction (PCR) leading to clusters representing the ‘intrinsic’ subtypes luminal A, luminal B, HER2-enriched, basal-like, and normal-like. Then, a minimized gene set was derived using the quantitative real-time PCR data for 161 genes. In a final step, the number of genes was further reduced, leading to a 50-gene set. This gene set was then analyzed for reproducibility of the subtype classification using algorithms like Prediction Analysis of Microarray (PAM). This PAM50 assay can be performed using formalin-fixed paraffin-embedded (FFPE) material. It recapitulates intrinsic subtypes as discrete entities and provides a risk of relapse (ROR) score with independent prognostic significance in multivariate analysis. The clinical meaning of intrinsic subtypes was further refined by incorporating clinical information. This combined model (subtype and tumor size) was a significant improvement compared to both the clinicopathological model and the intrinsic subtype model alone. In addition, subtypes and risk score were also used to assess the likelihood of efficacy from neoadjuvant chemotherapy [17]. In order to obtain a high LoE, numerous prospective-retrospective studies were performed. These studies confirmed that PAM50 shows independent prediction of outcome in breast cancer [18–20]. Especially in ER-positive breast cancer treated with endocrine therapy, PAM50 is able to separate distinct prognostic groups, with the ROR low category showing a 10-year metastasis risk of < 3.5% [21]. Additionally, PAM50 ROR score and ROR-based risk groups can differentiate ER-positive breast cancer patients treated with endocrine therapy with respect to their risk for late distant recurrence [22]. The low-risk group had a risk of late metastasis of 2.4% as compared with 17.5% in the high-risk group. These results could help in counseling patients who could be spared or potentially benefit from extended hormonal therapy beyond 5 years of treatment.

Meanwhile, Prosigna® (NanoString Technologies, Seattle, WA, USA), a PAM50-based subtype classifier and risk model on the Na-
noString nCounter® Dx platform, is used for decentralized testing in clinical laboratories [23]. Results from the training and validation data sets showed that this assay provided an accurate estimate of the risk of distant recurrence in hormone receptor-positive breast cancer consistent with the previously published PCR-based PAM50 assay. To ensure reproducibility in a decentralized setting, Nielssen et al. [24] validated the analytical performance using FFPE breast tumor specimens across several clinical laboratories. Today, therapeutic decisions are based on subtyping of breast carcinomas (table 3) [2]. Since the originally described intrinsic subtypes require molecular testing, surrogate approaches have been developed using more widely available immunohistochemical tests for ER and progesterone receptor together with immunohistochemistry or in situ hybridization tests for HER2. For separation of luminal A and luminal B breast cancer, Ki-67 is frequently used as an immunohistochemical marker of proliferation. However, one has to keep in mind that reproducibility is far from satisfying showing high inter- and intra-observer variability [25]. Bastien et al. [26] examined the concordance of PAM50 breast cancer subtyping with standard immunohistochemical markers. 814 tumors from the GEICAM/9906 phase III clinical trial were analyzed both with immunohistochemistry and PAM50. The authors concluded that standard immunohistochemistry did not adequately identify the PAM50 gene expression subtypes. Indeed, the current St. Gallen consensus noted increasing evidence for the prognostic value of commonly used multiparameter molecular markers [2].

In an attempt to simplify breast cancer subtype prediction, Haibe-Kains et al. [27] determined the expression of 3 genes (ER, HER2, aurora kinase A (AURKA)) in a large cohort of publicly available gene expression datasets (n = 5,715). They showed in their comprehensive analysis that adequate classification of the major and clinically relevant molecular subtypes of breast cancer can be robustly achieved with quantitative measurements of these key genes. However, these findings were challenged by others claiming that the PAM50 assay explained a significantly greater amount of gene expression diversity and that classification of the major and clinically relevant molecular subtypes of breast cancer was best captured using larger gene panels [28].

### Table 2. Commercially available molecular tests

<table>
<thead>
<tr>
<th>Provider</th>
<th>Type of assay</th>
<th>Type of tissue</th>
<th>Central lab</th>
<th>Indication and population studied</th>
<th>Analytical validation</th>
<th>Clinical validation</th>
<th>Prospective-retrospective evidence (recruited patients, %)</th>
<th>Prospective evidence (pending)</th>
<th>CTS</th>
<th>LoE2009</th>
<th>AGO recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MammaPrint*</td>
<td>Agendia 70-gene assay</td>
<td>fresh frozen or FFPE DNA microarrays</td>
<td>yes</td>
<td>prognostic N0-1</td>
<td>yes</td>
<td>yes</td>
<td>multicentric validation</td>
<td>MINDACT</td>
<td>C</td>
<td>I</td>
<td>+*</td>
</tr>
<tr>
<td>Oncotype DX*</td>
<td>Genomic Health 21-gene recurrence score</td>
<td>FFPE quantitative reverse transcription PCR</td>
<td>yes</td>
<td>prognostic N0-1, ER+</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>TAILORx, RxPONDER, ADAPT</td>
<td>B</td>
<td>I</td>
<td>B</td>
</tr>
<tr>
<td>EndoPredict*</td>
<td>Sividon 11-gene assay</td>
<td>no prognostic N0-1, ER+/HER2-</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>MA.12 (49), MA.5 (66), ABCSG 8 (40), ATAC (16), GEICAM/9906 (66)</td>
<td>B</td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Prosigina*</td>
<td>NanoString 50-gene assay</td>
<td>NanoString nCounter®</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>MA.12 (49), MA.5 (66), ABCSG 8 (40), ATAC (16), GEICAM/9906 (66)</td>
<td>B</td>
<td>I</td>
<td>II</td>
</tr>
</tbody>
</table>

AGO = Arbeitsgemeinschaft für Gynäkologische Onkologie; CTS = category of tumor marker studies; FFPE = formalin-fixed paraffin-embedded; PCR = polymerase chain reaction; ER = estrogen receptor; LoE = level of evidence; +* = should only be used in selected patients if all other criteria are inconclusive for therapeutic decision making.

### Table 3. Systemic treatment recommendations according to subtypes [2]

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Type of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A-like</td>
<td>endocrine therapy alone according to menopausal status</td>
</tr>
<tr>
<td>Luminal B-like</td>
<td>endocrine therapy appropriate for menopausal status plus adjuvant cytotoxic chemotherapy in many cases</td>
</tr>
<tr>
<td>Estrogen receptor (ER)-positive and HER2-negative</td>
<td>chemotherapy plus trastuzumab plus endocrine therapy appropriate for menopausal status</td>
</tr>
<tr>
<td>ER-negative and HER2-negative</td>
<td>chemotherapy plus trastuzumab</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>cytotoxic chemotherapy including anthracycline and taxane</td>
</tr>
</tbody>
</table>

In an attempt to simplify breast cancer subtype prediction, Haibe-Kains et al. [27] determined the expression of 3 genes (ER, HER2, aurora kinase A (AURKA)) in a large cohort of publicly available gene expression datasets (n = 5,715). They showed in their comprehensive analysis that adequate classification of the major and clinically relevant molecular subtypes of breast cancer can be robustly achieved with quantitative measurements of these key genes. However, these findings were challenged by others claiming that the PAM50 assay explained a significantly greater amount of gene expression diversity and that classification of the major and clinically relevant molecular subtypes of breast cancer was best captured using larger gene panels [28].

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Those validation studies showed that the 70-gene signature added prognostic significance in older patients between 55 and 70 years of age [31] as well as in node-positive patients with 1–3 [32] and even 4–9 [33] involved lymph nodes. A first prospective evaluation of MammaPrint was presented in the observational microRay-prognoStics-in-breast-cancer (RAS-TEK) study [34]. This study enrolled 427 patients, and confirmed the additional prognostic value of the 70-gene signature to clinicopathological risk estimations such as Adjuvant! Online. However, even though patients were enrolled prospectively, the final choice of therapy was not only based on the results of the 70-gene signature but also on Dutch guidelines of 2004 and doctor and patient preferences. To overcome these limitations, a randomized prospective study is necessary. To achieve the highest LoE, MammaPrint was also prospectively investigated in the randomized Microarray In Node negative Disease may Avoid ChemoTherapy (MINDACT) trial [35]. Results of this trial are still pending. A substantial limitation of the original 70-gene signature was that it needed fresh-frozen tumor tissue. To improve feasibility, a transfer of this signature to FFPE tissue was necessary. After method optimization, the performance of MammaPrint using FFPE tissue was assessed in an independent series of matched tissue from 5 hospitals (n = 211) [36]. MammaPrint was successfully translated to FFPE tissue with high precision (97.3%) and repeatability (97.8%). The authors concluded that FFPE results were equivalent to results derived from fresh tissue.

Oncotype DX®

The RS (Oncotype DX®, Genomic Health, Redwood City, CA, USA) is a well-known and broadly used assay using FFPE tissue. This reverse transcription PCR assay measures the expression of 21 genes in RNA extracted from FFPE samples of tissue from primary breast cancer. This test was developed specifically for patients with ER-positive node-negative breast cancer treated with adjuvant tamoxifen [37]. Kaplan-Meier estimates of the rates of distant recurrence at 10 years in the low-risk, intermediate-risk, and high-risk groups were 6.8, 14.3, and 30.5%, respectively. In a multivariate Cox model, the RS was independent of age and tumor size (p < 0.001). Thus, the RS was shown to predict distant recurrence in tamoxifen-treated patients with node-negative ER-positive breast cancer.

Expectably, the RS helps to identify patients with little additional benefit from adjuvant chemotherapy [38]. In the National Surgical Adjuvant Breast and Bowel Project (NSABP) B20 trial, patients were randomized to tamoxifen alone versus tamoxifen + chemotherapy. Patients with a high RS (≥31) had a large benefit from chemotherapy with a relative risk (RR) of 0.26, 95% confidence interval (CI) 0.13–0.53, and an absolute decrease in the 10-year distant recurrence rate of 27.6%. Patients with a low RS (<18) derived minimal, if any, benefit from chemotherapy (RR 1.31, 95% CI 0.46–3.78, and an absolute decrease in distant recurrence rate at 10 years of 1.1%). Patients with an intermediate RS did not have a large benefit from the addition of adjuvant chemotherapy. The authors concluded that the RS predicts the magnitude of chemotherapy benefit in node-negative ER-positive breast cancer patients. The independent prognostic and predictive significance of the RS was also confirmed in endocrine-responsive node-positive patients receiving anthracycline-based chemotherapy [39]. The SWOG-8814 trial for postmenopausal women with node-positive ER-positive breast cancer showed that chemotherapy with cyclophosphamide, doxorubicin, and fluorouracil (CAF) before tamoxifen (CAF-T) added survival benefit compared to treatment with tamoxifen alone. The RS assay could be performed in 40% of the patients treated in this trial. There was no benefit of CAF in patients with a low RS (<18) (HR 1.02, 95% CI 0.54–1.93; p = 0.97), but an improvement in disease-free survival (DFS) for those with a high RS (≥31) (HR 0.59, 95% CI 0.35–1.01; p = 0.033). The test for interaction was positive in the first 5 years (p = 0.029), but not thereafter (p = 0.58). Albain et al. [39] concluded that the RS was prognostic for tamoxifen-treated patients with positive nodes and predicted significant benefit of CAF in tumors with a high RS but not for those with a low RS. However, the proposed benefit of chemotherapy should be viewed with caution, since both of these clinical trials (NSABP-B20 and SWOG-8814) included a proportion of HER2-positive patients. Since HER2 is also one of the genes measured in the RS, the results obtained for the RS may be partially due to the inclusion of HER2-positive tumors and might thus not be representative for its performance in the subgroup of ER-positive/HER2-negative tumors [40]. Nonetheless, in response to this justified concern, interaction between RS and chemotherapy benefit was reported to remain significant when corrected for HER2 status [41].

Since extended endocrine therapy (more than 5 years of tamoxifen) further reduced recurrence and mortality of breast cancer [42], there was growing interest in whether gene expression algorithms like Oncotype DX were able to predict the risk of late metastasis occurring after 5 years. Within the TransATAC study population, 665 patients with ER-positive node-negative breast cancer were analyzed [43]. In multivariate analysis, the RS predicted early distant recurrence (HR 1.80, 95% CI 1.42–2.29; p < 0.001) but not late distant recurrence (HR 1.13, 95% CI 0.82–1.56; p = 0.47). To further analyze the role for late recurrences, the 21-gene RS was assessed in 1,065 chemo- and tamoxifen-treated, ER-positive, node-positive patients from NSABP B-28 and 668 tamoxifen-treated, ER-positive, node-negative patients from NSABP B-14 [44]. For late
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Recurrences, the RS was strongly prognostic in patients with higher quantitative ER expression suggesting that extending tamoxifen beyond 5 years might be most beneficial in patients with a high (and intermediate) RS with higher quantitative ER expression and of limited benefit in patients with a low RS. Taken together, substantial prospective-retrospective evidence (LoE IB) shows that the RS assay is clearly prognostic in patients treated with endocrine therapy only. In an attempt to validate these results prospectively and obtain the highest LoE, the Trial Assigning Individualized Options for Treatment (TAILORx) enrolled 10,253 women with ER-positive, HER2-negative, and node-negative breast cancer. Patients with an RS < 11 were assigned to receive endocrine therapy alone without chemotherapy. Patients with an RS > 25 received endocrine therapy plus chemotherapy, and patients with an intermediate RS (11–25) were randomized to endocrine therapy +/- chemotherapy [45]. Recently, 5-year survival data of 1,626 patients (15.9% of the whole study population) who had an RS of 0–10 were published [46]. This low-risk group showed a very favorable outcome with endocrine therapy alone. At 5 years, the rate of invasive DFS was 93.8%, the rate of freedom from recurrence of breast cancer at a distant site was 99.3%, the rate of freedom from recurrence of breast cancer at a distant or locoregional site was 98.7%, and the rate of overall survival was 98.0%. Not surprisingly, these new prospective data of the Oncotype DX low-risk group very much supported the well-known prospective-retrospective data.

EndoPredict®

More recently, another gene expression assay using FFPE tissue was developed to assess the prognosis of early breast cancer patients treated with endocrine therapy [47]. The EndoPredict® (EP; Sividon Diagnostics GmbH, Cologne, Germany) risk score was developed using a supervised top-down approach focusing on genes correlated with distant metastasis. The final training set for the development of this risk score consisted of 964 ER-positive HER2-negative tumors from patients treated with adjuvant tamoxifen only in 4 German centers (Frankfurt, Hamburg, Mainz, Stuttgart). Starting from 253 fresh-frozen samples analyzed using Affymetrix HG-U133A arrays (Santa Clara, CA, USA), genes associated with 10-year distant DFS were selected using a top-down approach. In a first step, 22,283 probe sets as potential variables for prediction of distant recurrence were analyzed. In a second step, 104 candidate genes were selected using Cox regression analysis with time to distant recurrence as endpoint. In a pivotal step, transformation of results generated on fresh-frozen tissue employing Affymetrix arrays to a quantitative reverse transcription PCR-based format generated on FFPE tissue was done using 159 patients with paired fresh-frozen and FFPE sample material (Mainz). For 66 genes, high-performing primer-probe pairs were found. In a final step of the training procedure, the genes of interest were reduced to 8 cancer-relevant genes covering different biological processes such as proliferation, apoptosis, cell adhesion, cell signaling, and ER expression to obtain a multivariate algorithm based on normalized quantitative reverse transcription PCR values. This pre-specified algorithm was then validated independently in patients from 2 large randomized phase III trials (Austrian Breast and Colorectal Cancer Study Group (ABCSG)-6: n = 378, ABCSG-8: n = 1,324). This multigene EP risk score provided additional prognostic information on the risk of distant recurrence of breast cancer patients, independent of conventional clinicopathological parameters. The combination of EP with tumor size and number of involved lymph nodes (EPclin) resulted in 10-year distant recurrence rates of 4% and 4% in EPclin low-risk and 28% and 22% in EPclin high-risk patients in ABCSG-6 (p < 0.001) and ABCSG-8 (p < 0.001), respectively, and outperformed all conventional clinicopathological risk factors.

To investigate the reproducibility in a multicenter setting in different molecular pathology laboratories, round robin tests were performed [48]. The EP scores measured by the individual participants showed excellent correlation with the reference values (Pearson correlation coefficient of all values compared to the reference value 0.994). Most importantly, all samples were assigned to the correct EP risk group, resulting in a sensitivity and specificity of 100%, proving that the EP test was feasible for reliable decentralized assessment of gene expression in luminal breast cancer.

Additionally, EP improved the prognostic classification derived from common clinical guidelines like the National Comprehensive Cancer Center Network, German S3 and St Gallen 2011 guidelines in ER-positive HER2-negative early breast cancer [49]. EPclin reassigned 58–61% of women classified as high-/intermediate-risk (according to clinical guidelines) to the low-risk group with a 5% rate of distant metastasis at 10 years with endocrine therapy only. However, it should be taken into account that the exact definitions for luminal A (‘low-risk’) and luminal B (‘intermediate/high risk’) were slightly different in recent St. Gallen recommendations possibly leading to a lower proportion of patients classified as high-/intermediate-risk in 2015. Beyond that, EPclin is an independent prognostic parameter for both early and late metastasis [50]. EPclin classified 64% of the patients as low-risk with 1.8% late distant metastasis at 10 years of follow-up. These results give the opportunity to weigh side effects and competing health risks of extended adjuvant endocrine therapy against this favorable projected outcome. Interestingly, an exploratory analysis showed that expression levels of proliferative and ER signaling genes contributed differentially to the prediction of early and late metastasis. For early metastasis, proliferation-associated genes were most significant. In contrast, these genes lost prognostic significance in late metastasis. For the prediction of late metastasis, ER signaling genes were most significant. These results suggest differences in the underlying biology of early and late metastasis.

The prospective-retrospective evidence described above was achieved in postmenopausal ER-positive and HER2-negative patients. A recent publication investigated the prognostic impact in 555 ER-positive and HER2-negative tumors from the 800 available samples in the GECAM 9906 trial [51]. Patients in this trial were randomized to anthracycline-containing chemotherapy +/- paclitaxel followed by endocrine therapy. EP was prognostic not only in postmenopausal (HR 3.3, 95% CI 1.3–8.5; p = 0.0109) but also in premenopausal patients (HR 6.7, 95% CI 2.4–18.3; p = 0.0002), showing that the prognostic significance was not related to menopausal status.
Comparison of Different Gene Expression Signatures

To examine the concordance of different gene expression signatures in a large dataset of 1,380 ER-positive breast cancer patients treated with adjuvant tamoxifen only, Prat et al. [52] compared research-based versions of PAM50 intrinsic subtyping and ROR score, Oncotype DX, and MammaPrint [52]. These 3 signatures were consistently found to be independent predictors of relapse. Surprisingly, Cohen’s kappa coefficients between risk group assignments showed only slight fair agreement (range 0.11–0.42). Clearly, a head-to-head comparison of the prognostic accuracy of different signatures is of interest. Analyzing patients with ER-positive primary breast cancer treated with anastrozole or tamoxifen in the ATAC trial addressed this goal. Initially, Dowsett et al. [53] compared ROR scores in 1,017 patients from the TransATAC population using the NanoString nCounter and Oncotype DX. They concluded that the PAM50 ROR score provided more prognostic information than RS, with fewer patients being categorized as intermediate-risk and more as high-risk. Very recently, Dowsett et al. [54] presented a comparison between RS and EP as well as EPclin in 928 patients from the TransATAC population. Likelihood ratio tests were used to assess the prognostic information. The EP score significantly added prognostic information to the RS over 0–10 years and 5–10 years in node-negative as well as node-positive patients. EPclin and RS identified 58.8 and 61.7% of the patients as low-risk with a HR (95% CI) low- vs. non-low-risk of 5.9 (3.9–9.1) and 2.7 (1.9–3.8), respectively. Most strikingly, the lowest tertile of EPclin in node-negative patients identified a group with extremely good prognosis and a risk for distant recurrence of 0.5% after 10 years of follow-up.

In conclusion, intrinsic subtypes and gene expression have substantially improved the understanding of the biological diversity and heterogeneity of breast cancer. Especially in ER-positive and HER2-negative patients, a reliable estimation of the risk of recurrence is crucial to select the appropriate adjuvant therapy. At present, the AGO recommended these multigene assays described above with +4, meaning that these tests should only be used in selected patients if all other criteria were inconclusive for therapeutic decision making.

Disclosure Statement

M. Schmidt reports personal fees from Astra Zeneca, Celgene, Eisai, Janssen, Novartis, Pfizer, Pierre-Fabre, Roche, Sividon, TEVA. In addition, M. Schmidt has patents regarding prediction of chemotherapeutic response in breast cancer and molecular markers for breast cancer prognosis pending. The other authors have nothing to disclose.

References

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