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Genomic Profiling in Luminal Breast Cancer

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Summary
The developments in gene expression analysis have made it possible to sub-classify hormone receptor-positive (luminal) breast cancer in different prognostic subgroups. This sub-classification is currently used in clinical routine as prognostic signature (e.g. 21-gene Onoctype DX®, 70-gene Mammaprint®). As yet, the optimal method for sub-classification has not been defined. Moreover, there is no evidence from prospective trials. This review explores widely used genomic signatures in luminal breast cancer, making a critical appraisal of evidence from retrospective/prospective trials. It is based on systematic literature search performed using Medline (accessed September 2013) and abstracts presented at the Annual Meeting of American Society of Clinical Oncology and San Antonio Breast Cancer Symposium.

Zusammenfassung
Fortschritte bei Genexpressionsanalysen erlauben eine Einteilung des Hormonrezeptor-positiven (luminalen) Mammakarzinoms in verschiedene prognostische Untergruppen. Diese Subklassifikation wird in der klinischen Routine als prognostische Gensignatur (z.B. 21 Gene/Oncotype DX®, 70 Gene/Mammaprint®) genutzt. Die optimale Methode zur Sub-Klassifizierung ist jedoch noch nicht definiert. Bisher fehlt die Evidenz prospektiver Studien. Diese Übersichtsarbeit analysiert die am weitesten verbreiteten genomischen Signaturen im Sinne einer kritischen Bestandsaufnahme der aus retrospektiven/prospektiven Studien verfügbaren Evidenz. Diese Analyse basiert auf einer systematischen Literaturrecherche mittels Medline, sowie der bis September 2013 veröffentlichten Abstracts beim Annual Meeting of American Society of Clinical Oncology und San Antonio Breast Cancer Symposium.

Introduction

Even with the significant progress in early detection and treatment of hormone receptor (HR)-positive (estrogen receptor (ER)- and/or progesterone receptor (PR)-positive) breast cancer (BC), it remains the most common female cancer in Western Europe, with at least 3 million newly diagnosed cases in 2010, and is still a potentially deadly disease. Currently, the major clinical challenges in HR^+ luminal BC are: (1) frequent overtreatment by adjuvant chemotherapy (CT) in about 70% of cases, based on classical clinical-pathological criteria (tumor size, nodal status, age); (2) selection of patients for extended endocrine therapy (ET) – it was recently shown that ET for 10 years is superior to that for 5 years [1]; and (3) finding predictive factors for targeted therapies that aim to overcome endocrine resistance [2].

The progress in high-throughput analysis of gene expression has led to a substantial change in our understanding of BC. Although HR status was already known as a predictive factor for ET efficacy and as a prognostic factor for BC, the work of Perou et al. [3], and Sorlie et al. [4] demonstrated that BC is a heterogeneous disease with distinct subtypes, but (sometimes) similar histomorphological characteristics. For the cDNA analysis (n = 8,102 genes), Perou and Sorlie used a small number (n = 40, and n = 78 in the second analysis) of fresh frozen BC samples. Hierarchical clustering analysis revealed 5 molecular subtypes that were relatively stable and intrinsic (mostly driven by genes of the same tumor).

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In addition, they were able to correlate these 5 molecular subtypes with differential patient outcome.

This work pointed to ER as a major factor in disease development and its subsequent biological and clinical characteristics. Most ER$^+$ cases can be assigned to the luminal A or B subgroup (named after the similarity of their gene expression with that of luminal cells of the normal breast gland). Proliferation-related genes seem to play a major role in the differentiation between both luminal subtypes. Low-proliferating luminal A tumors are associated with better prognosis and lower chemosensitivity than the high-proliferative, frequently poor grade luminal B subtype [4].

The molecular classification according to the definition of Perou et al. is often criticized because of the retrospective nature of the analysis, the small number of tumor samples used for the first analysis (n = 78), and the limited number of histological subtypes within small studies. Moreover, although the use of hierarchical clustering as a statistical method is widely applied in hypothesis-generating studies, it is not practicable for clinical use or prospective allocation of patients to the subtypes [5].

Molecular Subtypes

Allocation of tumors to either luminal subtype seems to be rather less dependable than that for basal-like and HER2 subtypes. Thus, if a nearest centroid predictor is used for allocation, many cases remain unclassified due to the low correlation with the subtype centroid [6]. Weigelt et al. [7] published a substantial lack of reproducibility of luminal subtypes using a single sample predictor method (similarity between subtype centroid and given case). There is only a moderate to weak correlation regarding luminal A or B subtypes between different datasets despite of a similar prognostic impact, thus underlining the mere research character of the molecular classification.

For clinical use, the PAM50 signature (as part of the Prosigna® test, NanoString Inc., Seattle, WA, USA) was developed as an mRNA quantitative reverse transcription PCR based on a 50-gene (and 5 reference genes) assay for sub-classification into the 4 molecular subtypes (luminal A/B, basal-like, and HER2). PAM50 is incorporated into a continuous Risk of Relapse (ROR) score [8], which includes the weighting of proliferation genes within molecular subtypes with/without tumor size (ROR-S/-C). It allocates patients into 1 of 3 risk categories (low (< 10% distant relapses)/intermediate/high risk (> 20%) in N0 BC. This signature significantly increases the prognostic impact of the intrinsic subtypes alone [8], and has been shown to be prognostic while showing that the outcome of the luminal B subtype may be quite comparable to basal-like and HER2 subtypes. The prognostic impact of ROR/PAM50 was confirmed in several retrospective analyses of prospective studies. In patients treated by tamoxifen

Table 1. Summary of genomic signatures

	PAM50/ Prosigna/ Risk of recurrence (ROR)	Oncotype Dx/ Recurrence score (RS)	Mammaprint	EndoPredict (EP)	Genomic Grade (GG)	Breast Cancer Index (BCI)
Number of genes	50+5 reference genes	16+5 reference genes	70 genes	8+3 reference genes	97 genes	HOXB13/IL18BR ratio+5 genes (molecular grade)
Laboratory Method	Decentral RNA/RT-PCR on nCounter digital gene expression platform in FFPE tissue [21]	Central RNA/RT-PCR in FFPE tissue	Central RNA-microarray in FF and RT-PCR in FFPE tissue (60 genes) [22]	Decentral RNA/RT-PCR in FFPE tissue [23]	Central RNA in FF, and RT-PCR in FFPE tissue (6+3 reference genes) [24]	Central RNA in RT-PCR in FFPE tissue
Classification	Molecular subtypes or continuous score (ROR+/- tumor size) 0–100: L/IM/H:**: 0–40/41–60/>60 in N0 and 0–15/16–40/>40 in N1	Continuous score 0–100: L: (0–11/18); IM (11–25/18–30); H (>25/30)*	Two groups: L/H risk	Continuous score, reported as L/H risk Used as EpiClin (with tumor size and nodal status) by cut-off 3.3	2 groups: L/H (and equivocal) risk	Continuous score 0–10: 3 groups L/IM/H-risk
Development	Microarray/RT-PCR analysis of 1,906 genes in 122 FF tumors from 189 individuals to correlate with molecular subtypes, selection of 161 genes than minimalization to 50 genes; ROR was developed on 141 untreated N0 patients from NKI, later validation on published data sets (n = 620 untreated N0/N+) [8]	RT-PCR analysis of 250 genes from literature, later their analysis in FFPE from 447 heterogeneously treated patients (N0; partly from NSABP B-20/N+), than correlation with outcome	Microarray analysis of 5000 genes in FF of 78 patients < 55 years old with known clinical outcome (distant relapse vs. no after 8 years follow up); 231 genes were associated with prognosis, 70 genes used for the signature [25]	Selection of 104 candidate genes for ER$^+$/HER2$^-$ (out of 4,187) from 253 tumors (FF, Tam-treated) to correlate with clinical outcome; reduction to 63 genes for FFPE, later training in n = 930, selection of 8 genes	Microarray analysis in 31 G1 and 33 G3 ER$^+$ tumors to find 97 genes associated with grade, validation and survival analysis in n = 597 samples from published cohorts (incl. NKI) [26]	Microarray analysis in 60 ER$^+$ FF tumors Tam-treated with known outcome, identification of 19 genes→ 2 genes, analysis in further 20 FFPE samples [27]; MGI: analysis of 39 genes associated with G3, correlation in publicly available cohort (n = 251) and reduction to 5 genes validation and translation into RT-PCR (n = 408) [28]

Table 1 continued on next page.

Table 1. continued

	PAM50/ Prosigna/ Risk of recurrence (ROR)	Oncotype Dx/ Recurrence score (RS)	Mammaprint	EndoPredict (EP)	Genomic Grade (GG)	Breast Cancer Index (BCI)
Prognosis	TransATAC (n = 1,017/ 5,880): postmenopausal N0/N+; ER+, ET-treated; 58–61% L risk [29] ABCSG-8 (n = 1,478/3,714) postmenopausal N0/N+, ER+, ET only 34% L risk [15], MA-12 (n = 398/672) N0/N+, ER+/- premenopausal Tam or nihil-treated, all CT; 34% LumA [10]; MA-5 (n = 476/716) premenopausal N+ patients, ER+/-, treated by CT; 32% LumA [12], GEICAM 9906 (n = 820/ 1,246) N+, ER+/-treated by CT: 34% LumA (56% of ER+/HER2-) [30] C9741 (n = 1,321/2,005) N+, treated by (dose-dense) CT 32% LumA	NSABP B-14 (n = 668/ 1,404) N0, ER+, Tam-treated 51% L risk [31, 32]; NSABP B-20 (n = 651/ 1,404) N0, ER+, Tam vs. CMF-Tam [33]; TransATAC (n = 1,230/ 5,880) 59–60% L risk [29, 31] SWOG-8814 (n = 367/ 927) ER+ postmenopausal N+, Tam or CEF-Tam treated, 40% L risk [34] E2197 (n = 465/2,885) ER+, N0/N1, EC or ET treated, 46% L risk [35] NSABP B-28 (n = 1065/ 3060) ER+ N+, EC or EC-paclitaxel treated; 36% L risk [36]	Retrospective validation in 295 N0 (including test set) /N+ T1/T2 patients < 53 years old; 37% CT treated, 39% L risk [37] Retrospective validation n = 302 N0 < 60 years old, CT-untreated, 37% L risk [38] Retrospective, n = 241 N1, 50% CT-treated, 41% L risk [39] Retrospective, n = 148 N0 mostly untreated postmenopausal 66% L risk [40] Prospective observational N0 n = 427, 51% L risk [41]	ABCSG 6 (n = 378/2021) and 8 (n = 1324/3714) postmenopausal N0/N+, ET-treated: 48–51% L-risk [42, 43] GEICAM 9906 (n = 800/ 1,246) N+, ER+, treated by CT: n = 555 ER+/HER2-, 25% L risk [44]	Retrospective validation study, n = 249 N0/N++, treated by ET and n = 417 untreated, 56–60% L risk [45] PACS 01 (n = 204/200) N+, treated by CT, 53% L risk BIG 1–98 (n = 883/ 4,922) postmenopausal N0/N+, ET-treated, 36% L risk [46] EC-Doc (n = 776/2,011) N1 CT-treated, 14% L risk in HR+ [47]	2 gene ratio: NCCTG 89–30–52: (211/256) treated by Tam, prognostic only in N0 [48] BCI: Stockholm trial (n = 588/2,738) postmenopausal ER+, N0 and tumors < 3 cm, Tam-treated and untreated, 57% L risk [49] TransATAC n = 66%/5,880) N0, ER+, no CT postmenopausal, 58% L risk [50]
Prediction adjuvant CT	No	Yes NSABP B-20 (N0) Hr for ET vs. CT-ET in L risk = 1.31, in H risk = 0.26) SWOG-8814: Hr ET vs. CT-ET in L risk = 1.02; in H-risk 0.59 (p = 0.03)	Meta-analysis of retrospective studies (n = 541 with ER+; ET (n = 315) vs. ET-CT treated (n = 226): Hr ET vs. CT-ET = 0.26 (n.s.) in L risk and Hr = 0.35 in H risk (p < 0.01) [51]	No	No	No
Prediction NACT Benefit	Yes (prospective) [52]	Yes (retrospective) [53]	Yes [52, 54]	No	Yes (retrospective) [55]	No
Prediction ET Benefit	Yes	Yes	No	No	No	Yes (extended ET) [56]
Late relapse	Yes [15, 16]	No	No	Yes [57]	No	Yes [58]
Local relapse	Yes [59]	Yes [54, 60]	Yes [61]	No	No	No
Prognostic in M1	Yes (retrospective data) [62]	Yes (prospective) [44]	No	No	No	No
Prospective studies	Secondary analysis RxPonder trial	TAILORX (n > 10,000 in N0 ER+) R: ET vs. ET-CT in RS 11–25 RxPonder (n = 4,000 in N1,: R: ET vs. ET+CT in RS 0–25 Observational PlanB: High clinical risk N0/N1, no CT in RS 0–11 (n = 318) ADAPT (n = 4,000), no CT in RS 0–11, CT in RS 12–25 depend on neoadjuvant ET response (by Ki-67)	MINDACT (n = 6627), R ET vs. CT-ET in clinical vs. genomic discordant cases Observational prospective raster (n = 427, 80% HR+) [54]	No	ASTER 70 s (n = 2000) GG as CT decision in women > 70 years old	No
Guidelines (Recommendation for CT decision)	St. Gallen (prognostic) [63]	St. Gallen [63], NCCN [64], NICE, ASCO [65], AGO	St. Gallen (prognostic) [63]	St. Gallen (prognostic) [63]	No	No

FF = formalin-fixed, FFPE = FF paraffin-embedded, L/IM/H = low/intermediate/high, NKI = National Kanker Institut (Amsterdam), RT-PCR = reverse transcription polymerase chain reaction, ER = estrogen receptor, HR = hormone receptor, Hr = hazard ratio, MGI = molecular grade index, CT = chemotherapy, LumA = Luminal A, Tam = tamoxifen, C/E/F/M = cyclophosphamide/epirubicin/fluorouracil, methotrexate, ET = endocrine therapy, NACT = neoadjuvant CT

alone, the intrinsic subtype and ROR score were significant prognostic factors together with tumor stage [9]. Moreover, the luminal subtype determined by PAM50 was more predictive for tamoxifen benefit compared to ER status determined by immunohistochemistry (IHC) in premenopausal patients treated within the NCIC MA-12 study [10]. Later, Ellis et al. [11] showed similar activity for neoadjuvant ET in both luminal A/B subtypes. There is some published work on the predictive impact of PAM50 for anthracycline (in the HER2 subgroup) [12] and paclitaxel benefit – surprisingly in the low proliferation group [13] – next to a significant prognostic impact in these smaller retrospective analysis. However, despite strong evidence for a predictive impact of pathological complete response (pCR) within the neoadjuvant CT (NACT) setting, with a 2.3–5.3-fold increase in the pCR rate in the luminal B compared to the luminal A subtype [14], there is still no predictive data for the efficacy of CT + ET versus ET alone. The significant prognostic impact of ROR has recently been retrospectively validated within the TransATAC and ABCSG-08 postmenopausal collectives treated by ET alone. In both studies, ROR provided additional prognostic information beyond clinical variables for both early and late metastasis. Both luminal A and B subtypes and ROR score have shown a prognostic impact over both time periods (0–5 and 5–10 years) and provided information in addition to the clinical factors [15, 16]. A recently presented meta-analysis of the trials in N+ patients confirmed that a large proportion of N+ patients had a low risk (particularly patients with 1–2 positive lymph nodes) [17]. However, the lack of predictive data for the efficacy of adjuvant CT and extended ET, and of decision impact or health economic evaluation studies, are a major consideration for not recommending the PAM50 assay for routine clinical use at the moment. Currently, there are already 2 prospective observational studies of clinical outcomes for the NanoString® Technologies' Breast Cancer Intrinsic Subtype Test (BCIST), 1 in Spain run by GEICAM and 1 in Germany run by WSG (Women's Healthcare Study Group, *www.wsg-online.com*). Similar studies are planned with separate but also common analyses to look at the health economic impact in different reimbursement systems.

Whole Genome Sequencing

Besides the controversial discussion regarding standardization of the molecular classification, over the last few years the results of whole genome sequencing of tumors have been published [18]. The luminal subtype (in contrast to basal-like tumors) was shown to be characterized by an activated ER-FOXA1 complex. The luminal A subtype (defined by mRNA) harbored significantly more PIK3CA mutations than the luminal B subtype (45% vs. 29%), followed by more MAP3K1, and GATA3 and fewer TP53 mutations (12% vs. 29%). However, markers of PIK3CA activation were not elevated in luminal tumors (compared to basal tumors) [19]. GATA3 was also reported to be associated with the aromatase inhibitor (AI) response, and the MAP kinase gene MAP3K1 with both low proliferation levels pre- and post-AI treatment [11]. In the same paper, a significant difference in the number of point mutations was seen between AI-sensitive and -resistant cases.

In addition to PAM50, which aims to standardize the molecular classification of BC, several prognostic gene signatures have been developed for exact prognosis estimation, particularly in HR^+ BC (detailed in table 1). Most of the genes included are involved in proliferation [20], so that to understand their additional prognostic and/or predictive impact compared to classical clinical-pathological or IHC characteristics alone, a comparison with a central and independent pathology assessment of these factors is required.

IHC-Based Classification

Based on the assumption that proliferation is vital for the differentiation between luminal A and B subtypes, several attempts have been undertaken to replace gene signatures by surrogate IHC markers (e.g. ER, PR, Ki-67, HER2) and/or histological grade assessment [21]. Cheang et al. [87] published a Ki-67 cut-off of ~14% as an optimal discriminator between luminal A and B subtypes (sensitivity 72%; specificity 78%). Therefore, high Ki-67 and/or positive HER2 status were proposed for the definition of luminal B tumors. The biological rationale behind this definition and a correlation with the intrinsic subtype led to the inclusion of the 14% Ki-67 cut-off into the St. Gallen guidelines in 2011, and to the proposed relative indication for a sequential chemoendocrine therapy in 'luminal B' tumors. However, there is still no consensus about the optimal measurement method and optimal cut-off of Ki-67 [22]. A significant inter-observer variability was confirmed in an international multicenter study (about 1/3 of patients were discordantly allocated to the luminal A vs. B subtypes) [23]. There are several retrospective analyses from prospective trials that confirm a strong prognostic impact of an IHC-based definition of the luminal A and B subtypes [24]. However, 2 recent studies have shown that Ki-67 as a continuous variable (as proposed by the International Consensus) and/or centrally measured grade (as a surrogate for molecular subtype) can provide comparable prognostic information to that derived from any genomic signature [25, 26].

Although several papers confirm a predictive impact of high Ki-67 with respect to an increase of pCR in the NACT setting [24], to a benefit from taxanes in addition to anthracyclines in the adjuvant setting [25, 27, 28] as well as to a benefit of NACT versus ET in the 'luminal B' subtype [29], so far no predictive effect of Ki-67 for benefit of adjuvant CT+ET versus ET alone has been shown [30]. This is difficult to understand in view of the prognostic impact of pCR particularly in

the luminal B-like but not in the luminal A disease [31]. Because of these methodological difficulties, the most recent St. Gallen Consensus Panel in 2013 voted for changing the Ki-67 cut-off to 20% (used by most groups for analysis) as an 'IHC-surrogate' definition of the luminal B subtype [32].

IHC scores such as IHC4, which uses centrally determined ER, PR, HER2, Ki-67, or Mammostrat [33], are highly effective methods for classifying HR$^+$ BC into prognostic subgroups. Dowsett et al. [34] published a similar prognostic impact of the IHC4 score compared to Oncotype DX® (Genomic Health Inc., Redwood City, CA, USA), and the ROR score. However, this could not be confirmed by other groups for the comparison of ROR versus IHC4 or IHC-based subtype [35]. Interestingly, Prat et al. [35] proposed the inclusion of PR > 20% into the luminal A definition. Other data from the WSG EC-Doc trial also reported a highly significant impact of PR expression within the luminal B subtype for the definition of 2 groups with distinct outcome (i.e. luminal B tumors with high PR expression have an outcome similar to the luminal A subtype) [36].

In summary, despite of methodical criticism, IHC-determined Ki-67, ER, and PR can be used in clinical routine primarily as a prognostic tool (but not as predictive tool for adjuvant CT), particularly if there is access to central pathology experienced in this field. Moreover, they seem to have an important impact as dynamic markers (e.g. drop of Ki-67 and/or loss of PR) and indicators for efficacy of ET, as they are currently used in the WSG ADAPT [37] and POETIC trials.

Prognostic and Predictive Multigene Signatures

The 21-gene Oncotype DX assay was developed on the basis of retrospective analyses across 3 studies of 447 patients (all N+ and N0). This analysis was followed by a validation study in tamoxifen-treated N0 ER$^+$ patients from the NSABP B-14 study [38]. Distant relapse (DR) was observed in 6.8% in the low- (L-) and in 30.5% of the high- (H-) risk groups by recurrence score (RS). A second validation study in N0 patients was conducted within the NSABP B-20 collective. There was no difference in DR after 10 years in the L- (-/+ CT: 96.8/95.6%) and intermediate- (IM-) risk groups (-/+ CT: 90.9/89.1%). The H-risk group derived a large benefit from adjuvant CT (10 year distant disease-free survival (distant DFS): 60.5 vs. 88.1%; hazard ratio = 0.26) [39]. 2 analyses compared the predictive value of RS and Adjuvant!Online with only high RS being a predictive marker for the benefit of adjuvant CT in N0 disease [39, 40] – a finding that was also previously shown in the context of NACT [41]. The prognostic impact of RS was also confirmed in N+ ET-treated populations in the TransATAC and SWOG-8814 trials (L vs. H risk: 17–40% vs. 49–57% DR after 9–10 years). The differences in the N+ subgroup were mostly driven by the cohort with 4 positive lymph nodes, who formed a rather high percentage of the population in the SWOG trial (38% vs. 21%) [42, 43]. The 10-year overall survival (OS) rates were equal in both studies (L vs. H risk: 77%/74% vs. 51%/54%). Nevertheless, the most important finding from this analysis was the predictive effect of RS for the benefit of CT-ET compared to ET alone in the RS H-risk group (hazard ratio = 0.59, p = 0.033), but not in the RS L-risk group (hazard ratio = 1.02, p = 0.97). None of the conventional prognostic factors were predictive for CT benefit by interaction analysis if RS was included. Curves for the benefit from CT (in the N1 and N2 subgroup) started to split at an RS of 20 (for the 0–5-year endpoint). In this study and in subsequent analyses, the prognostic and predictive impact of RS was stronger in the first 5 years of follow up than in years 5–10. There is further retrospective evidence from prospective trials, such as the E2197 [44] and NSABP B-28 studies, confirming a strong significant prognostic impact in addition to conventional markers, although no predictive impact with respect to paclitaxel CT was shown in the later trial [45].

Furthermore, several studies have indicated a prognostic impact of 21-gene RS, as shown by a good correlation between RS and loco-regional relapse (LRR). There was a very low LRR rate in the L- compared to the H-risk group, with: 1–4% versus 10–18%, respectively [46, 47]. A strong prognostic impact in primary metastatic ER$^+$ BC – as the last step of BC development – has recently been presented, showing a 2-year OS of 100% in the L-risk HR$^+$/HER2 group compared with 69% in the H-risk group (n = 70) [48].

Combination of Oncotype DX with further clinical factors (tumor size, age, grade), i.e. RS-pathological-clinical (RSPC), can increase the prognostic value of the test and reduce number of patients classified as L risk (with/without RSPC: 17.8% vs, 26.7%). However, it was less predictive by interaction analysis with regards to a CT benefit in the NSABP B-20 trial [40].

There are several decision impact studies from different countries evaluating the impact of RS in clinical routine. A change in CT decisions was reported in about 1/3 of patients in the pooled analysis, confirming L risk in 49% of cases (n = 1,437) [49]. RS has been evaluated in several randomized trials with first prospective evidence expected from the TailorX trial in 2015. This trial (2007–2011) aimed to confirm a 94% 5-year DFS in the L-risk group and investigate the CT impact in the IM risk group (RS 12–25) in N0 disease. The ongoing RxPonder trial is addressing the question of CT + ET versus ET alone in N1 L and IM risk by RS (RS 0–25) patients with N1 BC (n = 4,000). The WSG ADAPT trial is investigating the CT decision in the N0–1 IM risk by RS depending on early ET response (n = ~1,600 by ET alone). This is a follow-up trial to the planB trial (2009–2011) in which 318 patients with clinical IM or H risk and low RS opted for omission of CT.

The NCCN [50], ASCO [51], and current St. Gallen Consensus Panel [32] have recommended that the use of RS be

considered in the decision whether CT is given in ≥ pT1b N0 and N1mi disease.

Mammaprint® (Agendia NV, Amsterdam, The Netherlands) is a 70-gene prognostic signature that was developed in 2002, and has been recently been complemented by the BluePrint® (Agendia NV, for molecular subtype) and TargetPrint® (Agendia NV) assays (for ER, PR, HER2). Based on the expression of 70 genes, patients are allocated to the L- or H-risk subgroups, respectively (in both HR^+ and HR^- BC). A subsequent validation study (including patients from the original study) provided a clear correlation between risk assessment and relapse risk in 153 N0, mostly CT-naïve, patients (L vs. H risk: 13% vs. 56% after 5 years) [52]. A second validation study in N0 BC showed a 10-year OS in L- versus H-risk subgroups of 88% and 71%, respectively [53]. About 1/3 of patients were discordant by clinical and genomic assessment, so that CT could only be spared effectively in 20%. Later studies have shown a strong prognostic impact in N1 BC (n = 241, ~50% treated by CT; 5-year DFS 98% vs. 80% in L- vs. H- risk subgroups) [54], and also in postmenopausal N0 patients [55]. The 10-year LRR was reported as 5.7% versus 13.5% in the L- compared with the H-risk subgroups, respectively (n = 1,053) [56]. Knauer et al. [57] performed a meta-analysis of the predictive impact for adjuvant CT for 7 retrospective studies. The analysis showed a hazard ratio of 0.26 for 5-year DFS in the L-risk subgroup (ET vs. ET + CT: 93% vs. 99%, $p = 0.2$) and a hazard ratio of 0.35 (5-year DFS 76% vs. 88%, $p < 0.01$) in the H-risk subgroup. Meta-analysis data of prospective and retrospective studies [58] from the NACT setting showed a higher pCR rate (11% vs. 6%) after NACT in H- versus L-risk HR^+ patients, and a prognostic impact of pCR in H-risk disease but a better survival in the L-risk BC [59]. The prospective observational RASTER study revealed a better 5-year distant-recurrence-free survival (DRFS) in the L- compared to the H-risk subgroup (97% vs. 91.7%), which was independent of Adjuvant!Online allocation after 61 months of median follow-up [60].

The prospective randomized MINDACT trial, which directly compared genomic and clinical assessments, has been completed (n = 6,527). Discordance in risk assessment was reported in 32% of all cases, but CT could only be spared in 11.2% of all cases (per protocol) [61].

The EndoPredict® test (Sividon Diagnostics GmbH, Cologne, Germany) is another RNA-based tool for ER^+ BC that is widely used in German-speaking countries. It can be combined with clinical-pathological factors (tumor size and nodal status) into an EPClin score and divides patients into an L- or H-risk category even though the score itself is a continuous variable. The test was developed in mostly L-risk $ER^+/HER2^-$ patients receiving ET alone (65% N0, 10% G3). Pre-specified cut-offs were validated on samples from the ABCSG-6 and -8 studies, which included clinically L-risk postmenopausal patients (mostly G1/2, N0) treated by ET alone [62, 63].

At pre-specified cut-offs of EPclin, the 10-year risk for DR is 4% compared with 22–28% in the L- versus the H-risk group, respectively. The test identifies only 13–17% of 'luminal B' tumors as L risk, but provides additional prognostic information to clinical and IHC markers (Ki-67 and ER). It is reported to be prognostic for early as well as late recurrences. However, there is a distinct biology of early and late recurrences with the ER-related gene group (but not the proliferation group), which is associated with relapses in years 5–10 [64].

The test is listed by the St. Gallen Consensus [32] because of its strong prognostic data, but may not be recommended at the moment for the decision on CT in HR^+ BC due to the absent predictive data for adjuvant CT.

Genomic Grade® (GG, bioTheranostics Inc., San Diego, CA, USA) is primarily a 97-gene signature in HR^+ BC, which was published by Sotiriou et al. in 2006 [65]. The aim of the original work was to correlate known gene expression data with histological grade (HG) 1 and 3 tumors. Most of the differentially expressed genes were shown to be related to proliferation. Loi et al. [66] published a validation study, with a 10-year DRFS survival in GG1 of 83% in ET-treated N0/N+ patients. Later work demonstrated a better prognostic performance of GGI compared with Ki-67 IHC, mRNA, and mitotic count in a small cohort of N+ CT-treated patients from the PACS01 study (5-year DFS GG1 vs. GG3: 89% vs. 64%) [67]. Liedtke et al. [68] also reported predictive data for a better response to NACT but also worse survival in GG3 versus GG1 in HR^+ tumors (but not HR^- BC).

Two further prospective-retrospective studies were presented last year. Sotiriou et al. [26] compared the prognostic impact of GGI and Ki-67 in the BIG 1–98 monotherapy collective, with 40% of tumors identified as equivocal by GG analysis (39% pCR failure rate). In the N0 ET alone cohort, 99% a 10-year DFS was seen in GG1 compared to 87% in GG3 tumors. However, there was a comparable prognostic impact of both Ki-67 and GG as continuous variables in this cohort. Very similar data from the WSG EC-Doc study have also been reported (39% RNA failure rate; 76% GG3). GG was prognostic in multivariate analysis (including central grade), if used as a continuous, but not if used as a dichotomous, variable. IHC luminal B subtype was a better predictive factor for taxane benefit [25].

The Breast Cancer Index® (BCI, bioTheranostics Inc.) consists of the HOXB13/IL18BR gene expression ratio, which is prognostic [69] and predictive for ET benefit [70], and of the molecular grade index (MGI), which is a set of cell cycle-related genes. The gene expression ratio predicted outcome in untreated and in ET-treated patients better than conventional markers. The MGI, as a reflection of the 97-gene GG, was shown to provide complementary information to the GG [71]. The combination of both markers was validated in the Stockholm trial and a continuous BCI was established [72]. DFS at 10 years was 3% in the Tam-treated group and 50% in the untreated group. Interestingly, BCI was powerful in the de-

tection of both early and late recurrences as the only prognostic factor in the multivariate analysis, most likely due to the fact that it combines both ER- and proliferation-related factors [73]. This finding is of immediate clinical significance since, in a nested case-control study, HOXB13/IL18BR identified patients in the MA-17 trial who benefitted from extended ET by letrozole (83 recurrences vs. 166 non-recurrences) [74]. Identification of late recurrences was also assessed within the TransATAC trial. L-risk patients had a 10-year DR of 4.2% (compared to 17% of patients in the H-risk group with a 30% DR). Although the prognostic impacts of BCI, RS, and ROR were similar over the first 5 years, only BCI was predictive for late recurrences over the following 5–10 years.

Last but not least, the sensitivity to the ET index (SET) needs to be mentioned here as an example of second generation signatures. Symanns et al. [75] identified 165 genes co-regulated with ER. The signature is predictive of ER pathway activity and has a prognostic impact in patients receiving ET (with/without NACT, n = 523), but not in untreated patients (n = 341).

Concordance Between Prognostic Tools

The current St. Gallen Consensus recommends the use of multigene signatures particularly in luminal B disease for selection of patients who should undergo adjuvant CT [32]. As mentioned previously, there is only weak to moderate concordance between genomic tools and IHC classification of HR^+ BC. Prat et al. [35] reported k values of 0.2–0.41 for IHC versus PAM50 (in the earlier work k = 0.47 [9]), and 35–52% of IHC luminal B tumors were re-classified to luminal A using PAM50. It is important to state that the PAM50/ROR score provided additional prognostic information to that of the IHC subtype and IHC4, but the converse was not true. Similar data have been reported for the MINDACT trial, where only a 71% concordance between luminal subtypes by BluePrint and IHC (Ki-67 cut-off of 14%) was detected. In this study, 61% of 'IHC-based luminal B subtypes' were classified as L-risk luminal A by BluePrint [76].

The prospective planB study showed a comparable correlation between central grade, Ki-67 and RS (n = 2,566). 11% of luminal B (Ki-67 ≥ 20%) tumors were L risk and 48% IM risk by RS. The correlation between central grade and RS was poorer (k of 0.32) [77]. Only a moderate correlation of R_s = 0.65 was found between Oncotype DX and EndoPredict (Sividon Diagnostics GmbH, Cologne, Germany) in a small study, due mainly to 61% of H-risk patients by EndoPredict being classified as L or IM risk by RS [78].

Depending on different patient selection criteria, good concordance between H-risk assessments by the different genomic signatures seems to exist, but there are slightly divergent results in the L- and IM-risk groups. The prognostic value of a multigene assay is strongly dependent on the number of patients allocated into the IM-risk group and whether further clinical markers are included. Recently, Dowsett et al. [34] reported high concordance between L- and H-risk assessments (73% and 67%, respectively) obtained by ROR and RS. However, there was only a weak concordance in the IM-risk group (35%). Again, due to slightly different numbers in the IM-risk group, there was high prognostic concordance in the H- and L-risk groups, but less so in the IM-risk subgroups. ROR added some prognostic information to the RS, but the converse was not true.

Prat et al. [79] reported a consistent prognostic impact of all signatures (ROR, RS, Mammaprint, SET) as independent markers in the multivariate analysis (despite of a very low overlap between the individual genes, < 25%), so that a combination of these signatures increased the performance. ROR and RS showed the highest concordance. The results of the RxPonder trial are needed before final conclusions regarding a direct comparison between ROR-Score and RS can be drawn. This is a secondary objective of the trial.

Conclusions

The various genomic signatures presented in this review reflect the heterogeneity of HR^+ early BC and its distinct prognostic groups in a highly reproducible way. Valid analytical data are available for most of these signatures (e.g. for Oncotype DX, Mammaprint, EndoPredict, PAM50), and show: a high correlation between the same patients samples [80]; a high inter-laboratory correlation [53, 81, 82]; and good correlations between core biopsies and whole block samples [83, 84]. Although a multivariate prognostic effect of such genomic tools has been shown in most retrospective studies, it should be noted that several analyses did not include important prognostic variables such as Ki-67 and/or PR. Despite of their controversial reproducibility, inclusion of independently and centrally determined IHC factors is definitely required before a general recommendation to widely use genomic markers can be made. The combination with clinical markers seems to increase the prognostic value of these multigene tests [8, 40, 62], but may lead to a relative loss of their predictive impact.

To summarize the decision impact of genomic signatures, it is also important to remember that, despite a decision to change treatment in 30–40% of cases, absolute sparing of CT only occurred in about 10–13% of patients in most retrospective [85] and prospective [61, 86] studies. Combination of a genomic signature (Oncotype DX) with response to short-term preoperative ET within the WSG ADAPT trial [37] may increase this CT-sparing percentage up to 35–40% in patients with an originally strong indication for CT.

The IMPAKT 2012 Working Group Consensus Statement found the analytical and clinical validity of Oncotype DX and

Mammaprint convincing. However, none of the tests has shown robust clinical utility so far. Results of prospective trials (TAILORX, RxPOnder, MINDACT, planB, ADAPT) are eagerly awaited for clarification of the clinical impact of genomic tools within HR+ BC. Oncotype DX is recommended by the 2013 St. Gallen Consensus for making an adjuvant CT decision based on its predictive data. In Germany, the German Arbeitsgemeinschaft Gynäkologische Onkologie (AGO) still recommends the use of molecular tools only in individual cases and within clinical trials. Nevertheless, the signatures discussed in this manuscript all have the potential to support clinical decision making in early BC, and thus to help both avoiding overtreatment by adjuvant CT as well as undertreatment.

Disclosure Statement

Oleg Gluz received honoraria (advisory board) from Genomic Health Inc. and Hanostring Inc. Daniel Hofmann has no conflicts of interest to disclose.

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