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The Clinical Research and Latest Application of Genomic Assays in Early-Stage Breast Cancer

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Abstract

Breast cancer is a kind of malignant tumor that seriously endangers women's life and health. Once diagnosed, most patients will receive a combination of treatments to achieve a cure. However, breast cancer is a heterogeneous disease. Even with the same clinical stage and pathological features, its response to treatment and postoperative recurrence risk may still be completely different. With the advent of genomic assay, some patients with early-stage breast cancer who originally needed treatment can still achieve long-term disease-free survival without adjuvant chemotherapy, so as to achieve personalized and accurate treatment mode to a certain extent. In this paper, we reviewed the 5 most widely used and studied genomic panel technologies in breast cancer, namely *Oncotype DX*, *MammaPrint*, *RecurIndex*, *PAM50,* and *EndoPredict*, according to accessibility and availability. Based on the results of the completed or ongoing clinical studies, we summarized the origin, applicable population, and clinical efficacy of each detection method, and discussed the potential development prospect of detection technology in the future.

Keywords

early-stage breast cancer, genomic expression profiles, *Oncotype DX*, *MammaPrint*, *RecurIndex*, prediction tools

Abbreviations

HR, hormone receptor; HER2, human epidermal growth factor receptor 2; BC, breast cancer; IDFS, invasive disease-free survival; IHC, immunohistochemistry; eBC, early-stage breast cancer; DRFS, distant relapse-free survival; ER, estrogen receptor; PR, progesterone receptor; LRR, locoregional recurrence risk; PMRT, postmastectomy radiotherapy; LRC, locoregional control; LRFS, local relapse-free survival; RFS, relapse-free survival; ROR, Risk of Recurrence; pCR, pathologic complete response.

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Introduction

Female breast cancer has surpassed lung cancer to become the main malignant tumor threatening women's health.[1] Nevertheless, its 5-year survival rate can still reach 90%, far higher than that of other cancers.[2] Hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative tumor are a subtype of breast cancer (BC) with good prognosis, which have an invasive disease-free survival (IDFS) of more than 10 years or even a lifetime without recurrence.[3,4] Comprehensive treatment according to the characteristics of tumor is the conventional method at present. However, the features revealed by clinicopathology indicators and immunohistochemistry (IHC) classifications are only a part of their external presentation and do not truly reflect the individual biological characteristics of the tumor itself. The differential expression of different genes between individual tumors may be one of the reasons for the formation of tumor heterogeneity. For patients with low clinical risk and an excellent prognosis, whether chemotherapy can be exempted while ensuring prognosis and survival has always been a controversial issue in clinical practice. Therefore, evaluating whether the tumor is inert or highly invasive

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through genetic detection can help to reduce or even eliminate chemotherapy in patients who are unlikely to benefit from this treatment. It can be said that genomic assays avoid the occurrence of overtreatment and undertreatment for early-stage breast cancer (eBC) to some extent.

Predictive studies on gene expression profiles have been carried out since 2002.[5–7] In recent years, multigene panels have played an increasingly important role in (neo)adjuvant treatment decision-making; they have been used to assess the likelihood of distant recurrence (DR) in 5 or 10 years among patients with HR+/HER2-tumors. When the clinical risk assessment is uncertain, molecular assays can play a decisive role in follow-up treatment. In the face of numerous and complex detection methods, 5 of the most frequently studied and widely used gene detection prognostic tools in breast cancer, namely *Oncotype DX®*, *MammaPrint®*, *RecurIndex®*, *PAM50®,* and *EndoPredict®*, were selected, based on the current clinical application of multigene detection tools. Compared with other types of genomic panels, they are mainly used in the clinical decision-making of postoperative adjuvant therapy for patients with eBC and have different characteristics and applicable groups. Therefore, we selected these 5 assays as candidates and summarized their origin and associated clinical trials (Table 1) to guide their application in different clinical populations.

Multigene Assay

21-Gene Recurrence Score (Oncotype DX®, ODx)

Oncotype DX® (Genomic Health) is one of the first clinically validated gene expression profiles to predict eBC prognosis. Based on genomic database and experimental analyses, Paik *et al* screened 21 genes associated with recurrence from 250 candidate genes in 2004.[12] The 21-gene consisted of 16 cancer-related genes and 5 reference genes (Table 2), which were analyzed to calculate a recurrence score (RS) ranging from 0 to 100. According to their RS, patients were divided into 3 risk groups (bound by 18 and 30) initially, which were associated with an 11% and 20% 10-year recurrence risk, respectively.[12,32] The higher the score, the greater the likelihood of recurrence and the benefit of chemotherapy. Currently, the 21-gene assay has been recommended by the guidelines as level 1A.

TAILORx was the first prospective study (N = 9719) to investigate the predictive value of the 21-gene assay in patients without lymph node involvement.[32] Previous studies[12–15,32] have shown that high-risk (RS≥26 or RS≥31) patients could benefit from chemotherapy, while low-risk patients (RS ≤10) have a slim possibility of developing DR over the next decade. The survival is unlikely to be affected by chemotherapy. According to the statistics, among patients with low clinical and genetic risk, the 5-year recurrence risk after endocrine monotherapy was no more than 1%, and the risk of any recurrence was no more than 2%.[3] After 9 years of follow-up, the distant relapse-free survival (DRFS) was still 96.8% in this group.[8] This conclusion has affected existing clinical practice. However, the benefit of chemotherapy in intermediate-risk patients (RS 11-25) remained unclear. Although TAILORx trial suggested that endocrine monotherapy was not inferior to chemoendocrine treatment in this group, further stratified analysis showed that chemotherapy had a better therapeutic effect in young women (≤50 years). With an increase of the 21-RS, the benefit of chemotherapy gradually emerged.[8] Simulation modeling revealed a similar result to that of TAILORx.[33] Among young patients with a RS 16-25, chemotherapy improved the 9-year DRFS by approximately 3%.[33] Nevertheless, this phenomenon may be the result of ovarian function suppression or damage caused by chemotherapy.

Although TAILORx trial identified chemotherapy beneficiaries based on the 21-RS, it did not take into account general characteristics such as age, comorbidities, and clinicopathological characteristics. The integration of clinical parameters and molecular scores is the key to improving the accuracy of prognosis assessment,[34] especially when the 21-RS results indicate moderate-risk and/or the underlying tumor features have an inconsistent prognosis compared with RS results. A study had indicated that only about 5% of the variability in the risk estimates provided by Adjuvant! Online (www.adjuvantonline.com) could be explained by the *ODx.*[16] The resulting RS-pathology-clinical (RSPC) assessment showed a higher predictive value than RS, further confirming the importance of clinicopathological parameters.[35] This new prognostic tool (RSClin) that integrated the 21-RS with clinicopathological relevant factors provided significantly more DR prognostic information than clinical model and RS model alone ($P < .001$).[36] In a modified simulation model, Jayasekera *et al* found 69% of elderly women with small tumors and moderate malignancy were at intermediate-risk and the absolute benefit of chemotherapy was less than 1%.[37] Even in the absence of the 21-RS, chemoendocrine therapy directly changed the 10-year risk of DR by only 1.3%. Conversely, for young women with the same pathological features, the absolute benefit of chemotherapy was relatively significant. The absolute benefit was 7.8% for patients with RS≥26. Therefore, it is still necessary to recommend genetic testing to evaluate the risk of recurrence for young breast cancer patients.

The RxPONDER study (N = 5083) explored the treatment options in patients with 1-3 lymph nodes involved and RS≤25.[11,38] Preliminary results were presented at the 2021 San Antonio Breast Cancer Symposium (SABCS) in the United States. According to this study, endocrine monotherapy can achieve a good clinical prognosis similar to chemotherapy in medium/low-risk postmenopausal women (91.9% vs 91.3%), while premenopausal women show good chemotherapy benefits (89.0% for endocrine-only vs 93.9% for chemoendocrine).[11] However, whether this result is related to the use of ovarian function suppression, resulting in major signaling pathway changes is unknown. In addition, further follow-up is needed to determine whether the recurrence score can consistently predict favorable chemotherapy outcomes.

70-Gene Signature Test (MammaPrint®)

MammaPrint® (Agilent), which was developed by the Dutch Cancer Institute in 2002,[6] was the first *in vitro* diagnostic multivariate index assay approved by the FDA in 2007.[39] The

Table 1. The Horizontal Comparison of Various Genomic Assays.

Genomic assays	Technique	Country and company	Prospective researches	Retrospective researches	Recommendations
***Oncotype DX*® (21-RS)**	qRT-PCR (16 cancer-related genes and 5 reference genes)	Genomic Health, Redwood City, CA, USA	TAILORx (2015, 2018)[3,8] WGS PlanB (2016)[9] ROXANE (2019)[10] RxPONDER (2021)[11]	NSABP B14 (2004)[12] NSABP B20 (2006)[13] E2197 (2008)[14] SWOG-8814 (2010)[15] TransATAC (2010)[16] NSABP B-28 (2017)[17]	ASCO, NCCN, ESMO, St. Gallen, NICE, AGO, CSCO
***MammaPrint*® (70-GS)**	cDNA microarrays (70 genes)	Agendia, Amsterdam, the Netherlands	RASTER (2007, 2013)[18,19] MINDACT (2016, 2021)[20,21]	TRANSBIG (2006)[22]	FDA, ASCO, CSCO, AGO, St. Gallen, NCCN, ESMO, AJCC
***RecurIndex*®**	cDNA microarrays (18 core genes and 10 auxiliary genes)	Simcere, JiangSu, China	NA	NA	NA
***Prosigna*® (PAM50-ROR)**	qRT-PCR nCoutner® (50 genes and 5 reference genes)	NanoString, Seattle, WA, USA	NCIC.CTG MA.5 (2012)[23] NCIC.CTG MA.21 (2015)[24]	ABCSG 8 (2014, 2015)[25,26] TransATAC (2015)[26]	FDA, ASCO
***EndoPredict*® (12-EP)**	qRT-PCR (8 genes and 4 RNA/DNA reference genes)	Sividon, Koln, Germany	GEICAM 9906 (2014)[27] ABCSG-34 (2020)[28] UCBG 2–14 (2020)[29]	ABCSG 6/ABCSG 8 (2011, 2013)[30,31] GEICAM 9906 (2014)[27]	NCCN, ASCO, AGO, ESMO, St. Gallen, AJCC, EGTM, NICE

Abbreviations: AGO, German Gynecological Oncology Group; AJCC, American Joint Committee on Cancer; ASCO, American Society of Clinical Oncology; CSCO, Chinese Society of Clinical Oncology; ESMO, European Society for Medical Oncology; FDA, Food and Drug Administration; NCCN, National Comprehensive Cancer Network; NICE, The National Institute for Health and Care Excellence; EGTM, European Group for Tumor Markers.

Table 2. The Genes of *Oncotype DX* Recurrence Score (RS).[12]

Cancer marker	Candidate genes
Proliferation	Ki-67, STK15, Survivin, Cyclin B1, MYBL2
Invasion	Stromelysin 3, Cathepsin L2
Estrogen	ER, PR, Bcl2, SCUBE2
HER-2	GRB7, HER-2
Others	GSTM1, BAG1, CD68
Reference	Beta-actin, GAPDH, RPLPO, GUS, TFRC

a. Algorithm: RS = + 0.47 × HER-2 group score - 0.34 × ER group score + 1.04 × proliferation group + 0.10 × invasion group score + 0.05 × CD68 − 0.08 × GSTM1 − 0.07 × BAG1.
b. Low risk (RS less than 18); intermediate risk (RS 18 to 30); high risk (RS more than 30).

technique uses DNA microarrays to analyze primary tumors and applies supervised classification to identify gene expression signatures indicative of poor prognostic characteristics. Of the 25 000 human genes extracted, approximately 5000 were significantly regulated. To improve accuracy, the optimal number of marker genes was ultimately reduced to 70 genes (Table 3), which were closely related to specific biological behaviors of tumor development.[6] It is worth noting that IHC markers, namely, estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67, were not included in this genome analysis.

In 2006, results from a multicenter retrospective study, TRANSBIG, had firstly validated the clinical benefits of 70-gene panel in node-negative breast cancer.[22] A large sample of prospective MINDACT study (N = 6693), combined 70 gene signature and Adjuvant! Online, further confirmed that chemotherapy had no significant effect on 5-year DRFS of patients with high clinical risk and low genomic risk (95.9% vs 94.4%, $P = 0.27$, absolute benefit for chemotherapy was 1.5%).[20] The RASTER study compared 70 gene signature with 4 authoritative clinical risk assessments.[18] The results showed that about one-third of patients had distinct outcomes of clinical risk and prognostic markers. However, there was no significant difference in 5-year DRFS and survival prognosis of patients who made clinical decisions based on clinical risk or genetic risk alone, but observably more patients could be exempted from chemotherapy after obtaining genetic risk information. According to statistics, 46.2% of patients with high clinical risk may avoid chemotherapy.[20] At the 2020 ASCO meeting, Cardoso *et al* presented an update on the MINDACT trial for the first time. At a median follow-up of 8.7 years, the 5- and 8-year DRFS in the nonchemotherapy group was not worse than that in the chemotherapy group (absolute benefits for chemotherapy were 0.9% and 2.6%).[21]

The latest exploratory study analyzed the prognostic effects of the 70-gene signature across patients with different lymph node statuses and age groups.[21] Regardless of whether lymph nodes were involved, the 8-year DRFS benefit induced by chemotherapy was minimal. Furthermore, with the extension of the follow-up time, potential clinically relevant differences based on age were observed. In the age-stratified analysis, the MINDACT trial reached similar conclusions to the TAILORx

Table 3. There Were Described the Biological Behaviors of 70 Genes Related to Tumor Progression and Metastasis and Classified the Distribution of Each Gene.[5,6,a]

Hallmarks of cancer	Specific gene distribution
Evading apoptosis	BBC3, EGLN1, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1
Self-sufficiency in growth signals	ESM1, IGFBP5, FGF18, SCUBE2, TGFB3, WISP1, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1
Insensitivity to antigrowth signals	TGFB3, FLT1, HRASLS, STK32B, RASSF7, DCK, MELK, EXT1, GNAZ, EBF4, MTDH, PITRM1, QSCN6L1
Tissue invasion and metastasis	COL4A2, GPR180, MMP9, GPR126, RTN4RL1, DIAPH3, CDC42BPA, PALM2, FLT1, TGFB3, IGFBP5, FGF18, WISP1, ESM1, SCUBE2, PITRM1, EXT1, EBF4, ECT2
Limitless replication potential	CCNE2, ECT2, CENPA, LIN9, KNTC2, MCM6, NUSAP1, ORC6L, TSPYL5, RUNDC1, PRC1, RFC4, RECQL5, CDCA7, DTL
Sustained angiogenesis	ALDH4A1, AYTL2, OXCT1, PECI, GMPS, GSTM3, SLC2A3, FLT1, FGF18, COL4A2, GPR180, EGLN1, MMP9
Miscellaneous	LGP2, NMU, UCHL5, JHDM1D, AP2B1, MS4A7, RAB6B
Unknown function	LOC100288906, C9orf30, ZNF533, C16orf61, SERF1A, C20orf46, LOC730018, LOC100131053, AA555029_RC

[a]The underline indicates that the gene has 2 or more biological behaviors.

Table 4. A Multigene Detection System Consisting of 18 Core Genes.[43]

Gene group	Core genes
Cell cycle and proliferation	DDX39, BUB1B, STIL, TPX2, CCNB1
Carcinogenic process	BLM, TCF3, PIM1, RCHY1, PTI1
Inflammation and immune response	CCR1, NFATC2IP
Cell-cell interaction	TRPV6, OBSL1, MMP15
Apoptosis	C16ORF7, DTX2
Metabolism	ENSA

*Algorithm: $4 \times \text{TRPV6} + 3 \times \text{DDX39} + 8 \times \text{BUB1B} + \text{CCR1} + \text{STIL} + 3 \times \text{BLM} + 11 \times \text{C16ORF7} + 4 \times \text{PIM1} + \text{TPX2} + 2 \times \text{PTI1} + 2 \times \text{TCF3} + \text{CCNB1} + \text{DTX2} + 2 \times \text{ENSA} + 5 \times \text{RCHY1} + 4 \times \text{NFATC2IP} + \text{OBSL1} + 2 \times \text{MMP15}$.

trial,[40] that no significant chemotherapy benefit was observed during follow-up in females over 50 years.[21] In other words, younger females may be more sensitive to the cytotoxic effects of chemotherapy itself, but it is unclear whether this benefit is due to the indirect endocrine effect of chemotherapy on ovarian function inhibition.[41] Obviously, the hypothesis that ovarian function suppression by chemotherapy indirectly affects prognosis has more support.[40,42] Whether ovarian function suppression can achieve the same benefit in these patients needs further clinical verification.

18-Gene-Based Clinical-Genomic index (RecurIndex®)

RecurIndex® (Simcere) is the only Asia population-based gene expression profile that has been validated in a large and diverse breast cancer population. Compared with *ODx*® and *MammaPrint*®, it is more suitable for Asian based on race and environment. This study measured the gene expression level of primary tumor specimens from 135 patients before receiving any treatment. Finally, 18 core genes with the highest correlation were identified from 258 potential genes that were closely related to breast cancer recurrence and metastasis (Table 4).[43] Algorithm is as follows: Among them, the expression of RCHY1, PTI1, ENSA, and TRPV6 was associated with good tumor biological behavior and disease control, while the expression of the other genes was closely associated with poor prognosis.[44]

The 18-gene prognostic score was first proposed by Cheng and his colleagues to predict locoregional recurrence risk (LRR) in patients with lymph node metastases and to identify patients who could avoid unnecessary postmastectomy radiotherapy (PMRT).[43–46] Studies have indicated that locoregional control (LRC) excessed 97% in low-risk patients, regardless of their level of lymph node involvement or they received PMRT. Conversely, those with intermediate-/high-risk LRR appeared to benefit from PMRT, and the higher the risk, the higher the rate of LRC after radiotherapy.[46] Additional study showed that 5-year local relapse-free survival (LRFS) was 3 times higher in low-risk patients than in those with high-risk. For low-risk patients, the 5-year LRFS was 100% with lymph node-negative, while that of high-risk patients was only 50.8%. Even when 1 to 3 lymph nodes are involved, the 5-year LRFS could still reach 95.2% in the low-risk population.[47] The 18-gene panel had a significant predictive value for 5-year DRFS.[44]

Clinicopathological factors represent the basic characteristics of tumors, and their role in the evaluation of recurrence and metastasis cannot be ignored, especially in patients with lymph node involvement. Combining clinical variables with inherited genes, 2 clinical-genome models, the LGM-CM4 (RI-LR, recurrence index for local recurrence) and DGM-CM6 (RI-DR, recurrence index for distant recurrence), emerged, to predict the risk of local recurrence and distant metastasis, respectively.[48] Study showed that the 10-year LRFS of the low-risk group even reached 100%, even without receiving PMRT, while it decreased to 79.2% in the

high-risk group. This finding may exempt low-risk patients with lymph node-positive from local radiotherapy. Furthermore, we found that the risk of relapse within 5 years was up to 100% in high-risk patients without chemotherapy and a nearly 20% risk of relapse within 10 years even after receiving adjuvant chemotherapy. These patients might consider intensive treatment to consolidate the effect. A 10-year follow-up study of DGM-CM6 (RI-DR) also found significant inter-group differences in DRFS and relapse-free survival (RFS).[49]

At present, domestic genomic assays are commercialized kits performed by gene companies. Apart from *MammaPrint*®, which has foreign original research authorization, *ODx* and other test technologies from Europe and the United States are not licensed. Therefore, the analysis results on the market are mostly uneven, and many domestic physicians prefer to adopt preventive chemotherapy. As a domestic-based gene analysis, *RecurIndex*® may become an upstart and provides a new reference for postoperative treatment.

Predictor Analysis of Microarray 50 (PAM50, Prosigna®)

Prosigna® (NanoString Technologies) is a fluorescent bar-based gene expression quantification technology,[50] which screened 50 subtype predictors out of 1906 intrinsic genes.[51] It was approved by FDA for the *in vitro* diagnosis of HR-positive eBC in postmenopausal women in September 2013. PAM50, a second-generation gene technology, was initially used to quantify gene expression and type of breast cancer tissues.[52,53] Compared with IHC staining of protein expression levels, the classification of cancer subtypes was quantified into specific expression values from the binary classification concept according to the expression matrix of mRNA levels,[23,54] providing better prognostic stratification and disease management strategies.[55]

Studies have shown that more than 30% of PAM50 tests for HER2-enriched showed negative results, although the inconsistency of ER/PR status between PAM50 and IHC was only appropriately 10%.[23,54] More than 20% of breast malignancies with HR+/HER2-negative by IHC were identified as HER2-enriched by PAM50 quantitative assay.[56] Thus, any endocrine therapy has little effect on prognosis in this population. However, there is also evidence that the inconsistency between PAM50 and IHC results may be due to differences in gene and protein expression caused by hormonal fluctuations during the menstrual cycle.[51,57] There are currently no large clinical trials to examine the effect of hormonal changes during the menstrual cycle on PAM50 typing.

With the development of second-generation sequencing technology, PAM50 was gradually applied to stratify postoperative recurrence risk,[23,54] namely, the effect of hormone therapy alone on prognosis in postmenopausal women. In node-negative patients treated with endocrine monotherapy, the *Prosigna*® Risk of Recurrence (ROR) score was even better than *ODx* at predicting prognosis, due to the addition of more clinicopathological information,[58] and also predicted the 10-years DR.[25,59,60] The risk of DR in postmenopausal women increased significantly with an increase in the ROR scores, but the annual hazard rates remained stable at less than 1% per year in the low-risk group as the follow-up time increased.[61]

The PAM50 provided valid prognostic information in patients with lymph node involvement as well, and the risk of 10-year DR increased nearly 3-fold in the high-risk group than another.[26,59] In patients with a low ROR score, regardless of lymph node status, the absolute 10-year DR was 4.3% after 5 years of endocrine monotherapy. However, it has also been suggested that clinical treatment score has a stronger prognostic function than ROR score in the lymph node–positive subgroup, while the ROR score seems to perform better in the lymph node-negative group.[61] The results are subject to further head-to-head comparison. In addition, Minya *et al* proposed that adding a 13-gene VEGF hypoxia signature might improve the risk stratification of the existing PAM50 assessment tool.[57] This feature has not been clearly verified.

Table 5. The Genetic and Molecular Score That *EndoPredict*® Contains.[30]

EP signatures	Included information
Proliferation-related genes	BIRC5, UBE2C, DHCR7
Hormone receptor-related genes	RBBP8, IL6ST, AZGP1, MGP, STC2
Normalization genes	CALM2, OAZ1, RPL37A
Residual genomic DNA	HBB
Clinical parameters	Tumor size, nodal status

12-Gene Assay (EndoPredict®, EP)

EndoPredict® (Sividon Diagnostics GmbH) is a successor to *ODx*; it is a second-generation molecular assay that has shown significantly superior to *ODx* in analyses. The risk score consists of 8 cancer genes and 4 regulatory housekeeping genes (Table 5).[30] *EP* was combined with tumor size and nodal status to construct a broader comprehensive risk scoring system (*EPclin*). The *EP*-scoring ranges from 0 to 15, separating low- and high-risk by 5, while the cutoff value for *EPclin* is 3.3, corresponding to a 10% chance of DR after 10 years.[30,62] The diagnostic kits for EP-scoring are currently on the market worldwide to assess the long-term prognosis for ER-positive patients and stratified the risk of recurrence to determine the need for combination chemotherapy or prolonged endocrine therapy in addition to endocrine therapy. Statistically, approximately one-third of patients adjusted their therapy decisions after *EPclin* detection.[63]

In nonchemotherapy patients, both *EP* and *EPclin* showed significant predictive value for the 5- or 10-year risk of DR,[30] making it the first test to distinctly increase common clinicopathological prognostic information.[31] Patients with high-risk were 5 times more likely to have a recurrence within 10 years than those with low risk,[30] and long-term follow-up showed that *EPclin* predicted recurrence independent of lymph node

status.[64] Therefore, regardless of lymph node involvement, low-risk patients were largely able to avoid chemotherapy, while high-risk patients face a 20% risk of relapse.[30,64] In comparison with other clinical guidelines, we found that less than 20% of women were classified as low-risk by the NCCN guidelines, the German S3 guidelines, and the St. Gallen criteria, while *EPclin* accounted for approximately two-thirds of the low-risk patients. Surprisingly, the outcomes of 10-year RFS were extremely similar. Thus, *EP*/*EPclin* can more accurately identify more patients with a very low probability of DR. Molecular stratification still has a strong predictive advantage in patients considered non-low-risk by clinical guidelines.[31,65] In the UCBG 2 to 14 study, 35.8% of patients at clinically moderate-risk changed treatment regimens after *EPclin* testing, 28.4% of whom had chemotherapy withdrawn.[29] In addition, *EP*/*EPclin* has also been proven to predict LRFS.[66] Fitzal *et al* noted that *EP* high-risk patients had a 30% increased LRR compared with low-risk patients. However, there are currently no tailored trials to guide local treatment after breast-conserving surgery in low-risk patients, such as type, dose, and frequency.

Discussion

In the era of precision and individualized therapy, molecular detection has become an indispensable part of cancer diagnosis, treatment, and prognosis prediction.[41] Gene expression characteristics have been validated to distinguish different prognostic population, but their potential functions do not appear to be fully exploited.

Genetic Assays and Reactivity to Neoadjuvant Therapy

Given their ability to optimize treatment options, gene expression characteristics are increasingly being used to identify candidate patients who are more likely to achieve a favorable pathological response to neoadjuvant therapy. Although the prognosis of HR+/HER2− breast cancer is significantly better than that of other subtypes, the pathologic complete response (pCR) rate of this subtype of breast cancer (7.5%-16.2%) is much lower than that of triple-negative or HER2 + BC (30%-50%).[67] This subtype may be insensitive to chemotherapy and has significant heterogeneity in the response to neoadjuvant therapy.[67] *ODx* is one of the earliest and most in-depth genetic tests. Luca *et al* first proposed that the 21-gene panel might predict neoadjuvant chemotherapy response in patients with locally advanced breast cancer in 2005.[68] Subsequent studies have confirmed that the 21-RS is significantly correlated with pCR, and those with high recurrence risk have a relatively high pCR rate.[69,70] Similar studies have been reported for *MammaPrint*®[71] and *EndoPredict*®.[28] In addition, a risk assessment study based on a 95-gene classifier developed in a Japanese population also suggested that high-risk individuals would have a higher benefit from neoadjuvant chemotherapy.[72] Neoadjuvant endocrine therapy appears to be a suitable option for low-risk patients.[73,74] Moreover, some trials have added cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) or mammalian target of rapamycin inhibitors to endocrine therapy and investigated whether 21-RS can be used as a biomarker to predict neoadjuvant efficacy.[75] The PLATO study is attempting to provide personalized neoadjuvant therapy to patients with luminal A breast cancer using the 70-gene assay in the hope of improving breast conservation rates.[76] The study is expected to be completed in 2025. Although all studies described homologous trends in predicting the response to neoadjuvant therapy in patients with the luminal A type, most lacked significant differences due to the limited number of patients enrolled in each study and the absence of clear inclusion criteria. The St. Gallen Consensus Conference approved the application of preoperative genomic analysis in patients with neoadjuvant therapy indications to optimize the type of surgery. Nevertheless, the ASCO guidelines do not recommend the use of gene expression assays to guide clinical decision-making for neoadjuvant therapy.[75,77] Therefore, further large sample analyses are still needed.

Genetic Assays and HER2-Positive and Triple-Negative Breast Cancers

Currently, most of the gene expression profiles available on the market are applicable to breast cancer patients with HR+/HER2 − disease. However, HER2-positive and triple-negative breast cancer have not been included in the applicable scope of downgrading therapy after testing due to their high degree of malignancy and strong invasive biological characteristics. The emergence of trastuzumab, a macromolecular anti-HER2-targeted therapy, has significantly extended IDFS in HER2-positive patients. Although the clinical prognosis of HER2-positive patients is relatively worse than that of luminal-type patients, there is heterogeneity in the biological behavior, prognosis, and therapeutic benefit of the former. Clinically, we can find patients with late recurrence and relatively good prognosis, as well as HER2-positive patients with recurrence and metastasis during the adjuvant period. Such prognostic uncertainty may be related to other related signaling factors in breast cancer. Perhaps we can classify these patients as having a higher or lower risk through polygenetic assays, and provide patients with a hierarchical systematic treatment that can be upgraded or downgraded based on further classification, such as enhanced targeted therapy or reduced chemotherapy dose and duration. In 2020, Aleix *et al* initially developed a multivariable prognostic score to guide the systematic treatment of early HER2-positive breast cancer and predict survival outcomes.[78] The PHERGain trial used ^{18}F-FDG-PET to identify patients with HER2-positive who might benefit from dual anti-HER2 therapy without chemotherapy.[79] The phase II trial TBCRC026 also attempted to predict the pCR rate after dual anti-HER2-targeted neoadjuvant therapy in HER2-positive patients using quantitative imaging.[80] As documented in the existing literature, genomic assays such as *ODx* have been used in studies to predict the response to neoadjuvant treatment of locally advanced breast cancer patients with HR +/HER2- disease.[28,73,74] Regarding neoadjuvant therapy, pCR

of HER2-positive and triple-negative breast cancer is much higher than that of HR+/HER2− breast cancer,[67] but there are still some patients with primary drug resistance. Perhaps these techniques can also be used to predict disease in HER2-positive or triple-negative breast cancers, which are considered clinically high risk, to identify potentially treatment-sensitive or drug-resistant patients. At present, the *RecurIndex*® 28-gene panel is also being explored in a phase II study to predict the efficacy of dose-intensive neoadjuvant chemotherapy for triple-negative breast cancer (ChiCTR2000034890). The final results have yet to be announced.

Genetic Assays and Ductal Carcinoma in Situ (DCIS)

With the popularization of screening technology, the detection rate of DCIS has increased over time. In contrast to invasive breast cancer, DCIS is usually asymptomatic. Although the recurrence risk of DCIS is very low and patients with DCIS can avoid chemotherapy and the damage caused by it, local radiotherapy and even total mastectomy are still unavoidable according to current guidelines. However, autopsy results have demonstrated that most cases of DCIS remain subclinical throughout their lifespan and do not cause a life-threatening condition. Follow-up schemes after DCIS detection may lead to overtreatment.[81,82] Among existing genetic tests, *ODx* can be used to predict the risk of local recurrence of DCIS, in addition to identifying low-risk patients with HR+/HER2− invasive breast cancer. The *ODx* breast DCIS score was also the first clinically validated and commercially available gene-expression assay.[83–85] Recently, a prospective study is being planned to evaluate whether a combination of clinicopathological criteria and the DCIS score can identify women with a very low risk of local recurrence after breast-conserving surgery who can avoid breast radiotherapy (NCT04797299). HR and HER2 status were not restricted. The results are expected in 2035. To the best of our knowledge, only the first-generation *ODx* test is currently available for breast cancer types other than invasive breast cancer. One study has shown that the ROR score also seems to have prognostic value for certain subtypes.[86] However, this finding requires further validation before application in clinical practice due to the small sample size. Perhaps in the coming years, tests that are commercially available or under development could be adapted for more types of breast cancer or even other cancers.

Conclusion

The advent of a variety of gene expression assays has created the possibility that patients with eBC may be exempted from adjuvant chemotherapy while gaining long-term survival benefits. Discrediting a widely accepted treatment is challenging, but in the history of BC research, such challenges are not uncommon. The changes from modified radical mastectomy to breast-conserving surgery and from axillary lymph node dissection to sentinel lymph node biopsy are successful precedents. Therefore, "subtraction" of chemotherapy to avoid excessive treatment is also an inevitable trend. The multigene prognostic analyses on the market are complicated, and the core genes included are not the same, which is bound to yield inconsistent results among gene tests. The results may be related to testing methods, techniques, tissue composition, or even race and environment. In the face of different results for the same sample, there will be deviations in the selection and judgment of clinicians. Therefore, these tests still need to be normalized and their scope of use is limited. Although some prognostic gene signatures are now available for inclusion in guidelines and commercialized for clinical decision-making, some experts believe that this technique has not been proven to meet the highest evidence-based standards and its predictive value is only wishful thinking.

Authors' Note

This is a review paper. No animal or human studies were conducted.

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