A narrative review of five multigenetic assays in breast cancer

Cheng Zeng[1,2], Jian Zhang[1,2]

[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Shanghai, China; [2]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

Contributions: (I) Conception and design: C Zeng; (II) Administrative support: Fudan University Shanghai Cancer Center; (III) Provision of study materials or patients: C Zeng; (IV) Collection and assembly of data: C Zeng; (V) Data analysis and interpretation: C Zeng; (VI) Manuscript writing: Both authors; (VII) Final approval of manuscript: Both authors.

Correspondence to: Jian Zhang. Department of Medical Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, No.270, Dong'an Road, Shanghai 200032, China. Email: zhangjian1001@csco.ac.cn.

Background and Objective: Breast cancer is a highly heterogeneous disease. Its incidence rate is increasing year by year and the mortality rate is the highest in female malignant tumors. Even patients with the same clinical stage and pathological grade have different response to treatment and postoperative recurrence risk. Although the prognosis of breast cancer in China has been gradually improved, there is still a certain gap compared with the 5-year survival rate as high as 89% in developed countries. In recent years, with the continuous enrichment of molecular sequencing data of breast cancer, gene detection technology has important reference value in prognosis judgement and guiding treatment of early breast cancer. This article reviews the current application and latest progress of genetic tests in comprehensive treatment for breast cancer, with a view to promote the precise treatment of breast cancer in clinical practice.

Methods: We conducted searches using the MeSH terms 'breast neoplasms' and 'genetic testing' in the PubMed databases from root to 22 January 2021. We conducted an additional search in the National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines to obtain additional information. The search was limited to English, Dutch, French and German articles and research involving humans. Out of the references screened, 51 articles were found eligible for inclusion finally.

Key Content and Findings: The article reviews the mechanisms and clinical trials of five genetic tests including Oncotype Dx, Mammaprint, Endopredict, mRNA expression of 50 genes (PAM50) and breast cancer index (BCI) in comprehensive treatment for breast cancer. All these tools have been proved to have prognosis value, but only two of them, Oncotype Dx and Mammaprint, are recommended as predictive tools for chemotherapy by National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO).

Conclusions: In order to promote the comprehensive treatment of breast cancer to "precision" and "individualization" for further development, people have extensively researched on multigene testing technology represented by Oncotype Dx, Mammaprint, Endopredict and mRNA expression of 50 genes (PAM50) and breast cancer index (BCI). Each of these five tools has its advantages and limitation, which must be weighed in a wise application.

Keywords: Breast cancer; gene expression profiles; genetic testing; precision medicine

Introduction

Mammary carcinoma is a heterogeneous disease, diversity of which is clear from the evaluation of gene expression, therefore, precise and individualized treatment is the only way which must be passed to improve the prognosis of breast cancer (1).

With the further development of molecular biology of breast cancer, the research on the receptor status of tumor cells has made considerable progress. By using immunohistochemistry and *in situ* hybridization, the three important receptors and proliferation index of breast cancer cells can be quantified, and then the molecular subtype of breast cancer can be achieved. According to the different combinations of the 4 indexes of estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2) and Ki-67, four different molecular subtypes of breast cancer were identified, namely luminal A, luminal B, HER2 positive type and three triple negative type, respectively. Although the application of molecular subtypes has successfully divided the breast cancer from a single disease into several important types, and has provided an important basis for diagnosis and decision-making, in fact, it still has a big gap from the real precise treatment, because a small number of subtypes of stratification system cannot accurately describe the huge heterogeneity caused by the large number of differential gene expression among tumors. For example, hormone receptor positive breast cancer patients are clinically at low risk, with good prognosis. The choice of endocrine therapy alone or endocrine combined with chemotherapy has perplexed the decision-making of clinical treatment regimen. After the introduction of multi-gene assay into clinical practice, through the recurrence risk score, some patients can avoid ineffective chemotherapy, which provides a decision-making basis for the formulation of clinical comprehensive treatment plan.

With the development of genomics, genetic testing has gradually entered clinical practice, thus making it possible for breast cancer to be precise and individualized, effectively avoiding the inadequate treatment or overmedicalization of breast cancer and further improving the prognosis of breast cancer (2).

According to St Gallen Consensus, at least five commercially gene expression profiles including Oncotype Dx, Mammaprint, Endopredict, PAM50, and BCI are obtainable, in which, Oncotype Dx and Mammaprint have been recommended for their predictive and prognostic value of chemotherapy based on National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines, while another three gene expression profiles can only provide prognostic value so far.

This retrospective review mainly figures out the development of five multi-gene assays and provides evidence on clinical validity and utility of these five profiles. *Table 1* shows the horizontal comparison of five genetic tests and differences among them more directly (*Table 1*). We present the following article in accordance with the Narrative Review reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-1920/rc).

Methods

We conducted searches using the MeSH terms 'breast neoplasms' and 'genetic testing' in the PubMed databases from root to 22 January 2021. In addition, reference lists of relevant articles were screened to identify key articles that had been missed. We conducted an additional search in the National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines. The search was limited to English, Dutch, French and German articles and research involving humans. Study selection took place in three stages: first, titles were reviewed, followed by a review of abstracts and then full texts. Articles without an available abstract were directly included in the full-text review stage. At each stage, a selection of over 150 articles was reviewed to reach consensus about applying the inclusion and exclusion criteria. We formulated inclusion and exclusion criteria. Articles were included when: (I) the article focused on breast cancer patients, and (II) the term 'genetic testings' or relevant synonyms were included. Articles were excluded when they focused on: (II) topics differing greatly from genetic testings and (II) other diseases. Out of the references screened, 51 articles were found eligible for inclusion finally. See the search strategy summary in *Table 2*.

Principles

Tumor tissues were locally sampled to extract RNA and then gene expression level was evaluated by the genetic test (32). All of currently available genetic tests are based on technologies of reverse transcription, using reverse transcriptase to convert messenger RNA (mRNA) into complementary DNA (cDNA). Then this cDNA library can then be used as the template for many different assays,

Table 1 Horizontal comparison among five genetic tests

Multigene test	Mammaprint	Oncotype DX	PAM50	Endopredict	BCI
Technique	DNA microarray	RT-PCR	RT-PCR	RT-PCR	RT-PCR
Samples	Fresh frozen tissues	FFPE	FFPE	FFPE	FFPE
Recurrence score	Genomic risk score	RS (0–100)	ROR (0–100), ROR-S; ROR-T, ROR-P; ROR-PT; intrinsic subtype	EP (0–15); EPclin	BCI (0–10)
Risk stratification	Low (score <0.4), high (score >0.4)	Low (RS <18); intermediate (RS, 18–30); high (RS >30)	Low; intermediate; high (details are given above)	Low (EP <5, EPclin <3.3); high (EP >5, EPclin >3.3)	Low (BCI <5.0825); intermediate; high (BCI >6.5025)
Clinical validity	van 't Veer (2002) (3); van de Vijver (2002) (4); TRANSBIG (2006) (5); RASTER (2007) (6); Mook (2009) (7)	NSABP B14 (2004) (8); ECOG2197 (2008) (9); TransATAC (2010) (10); SWOG8814 (2010) (11); WSG Plan B (2016) (12); NSABP B28 (2018) (13); RxPONDER (ongoing)	Parker (2009) (14); Dowsett (2013) (15); TransATAC + ABCSG 8 (2015) (16)	ABCSG 6/8 (2011) (17); EICAM/9906 (2014) (18); ABSCG 8 (2015) (19)	Ma (2008) (20); Zhang (2013) (21); Sgroi (2013) (22); NCIC MA.14 (2016) (23)
Clinical utility	MINDACT (2016) (24)	NSABP B20 (2006) (25); SWOG8814 (2010) (11); TAILORx (2018) (26)	DBCG 77B (2018) (27)	ABCSG 34 (2020) (28); UCBG 2-14 (2020) (29)	aTTom (2019) (30); Noordhoek (2020) (31)

PAM50, mRNA expression of 50 genes; BCI, breast cancer index; RT-PCR, reverse transcription and polymerase chain reaction; FFPE, formalin-fixed and paraffin-embedded tissues; RS, recurrence score; ROR, the risk of recurrence; ROR-S, ROR combined with subtypes; ROR-T, ROR combined with subtypes and tumor size; ROR-P, ROR combined with subtypes and proliferation; ROR-PT, ROR combined with subtypes, proliferation and tumor size; EP, the genetic score of Endopredict; EPclin, the combination of EP score and two clinical factors.

Table 2 The search strategy summary

Items	Specification
Date of Search (specified to date, month and year)	22 January 2021
Databases and other sources searched	PubMed; the NCCN and ASCO guidelines
Search terms used (including MeSH and free text search terms and filters).	'Breast neoplasms' and 'genetic testing'
Timeframe	Conducting searches using the MeSH terms 'breast neoplasms' and 'genetic testing' in the PubMed databases from root to 22 January 2021
Inclusion and exclusion criteria (study type, language restrictions etc.)	Articles were included when: (I) the article focused on breast cancer patients, and (II) the term 'genetic testings' or relevant synonyms were included. Articles were excluded when they focused on: (I) topics differing greatly from genetic testings and (II) other diseases
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Study selection took place in three stages: first, titles were reviewed, followed by a review of abstracts and then full texts. Articles without an available abstract were directly included in the full-text review stage. At each stage, a selection of over 150 articles was reviewed to reach consensus about applying the inclusion and exclusion criteria
Any additional considerations, if applicable	N/A

N/A, not applicable.

such as PCR or microarray-based gene expression profiles, which allows the examination of gene expression levels in the tumor tissues (32,33).

DNA microarray is a molecular biology technique for gene expression analysis (34). RNA is labeled with the fluorescent dye and hybridized against thousands of different nucleotide sequences corresponding to different genes on a solid surface. According to the fluorescence signal from a single location on the microarray, the gene expression level was estimated. It is worth noting that this technique requires high-quality RNA that can only be obtained from fresh tissues. Therefore, it cannot be applied to formalin-fixed and paraffin-embedded tissues (FFPE), thus, limiting the popularity of this technology (35).

Reverse transcription polymerase chain reaction (RT-PCR) is also a molecular biological technique combining reverse transcription and polymerase chain reaction (36). RT-PCR devices could measure fluorescence signals at defined points of each thermal cycle, so as to quantify PCR products in real time. Moreover, RT-PCR can provide highly accurate relative expression levels, which is much more convenient than DNA microarray.

The only gene expression profile using DNA microarray is Mammaprint, a 70-gene profile, because it needs fresh frozen tissues and can only be examined in the professional testing center, and its clinical application is restricted. Other gene expression assays on the basis of RT-PCR include the 21-gene Oncotype Dx, the 11-gene Endopredict, the 50-gene PAM50, and the 8-gene BCI. These four genetic tests mostly take samples of formalin-fixed and paraffin-embedded tissues (FFPE) from surgical resection or core biopsy.

Development and risk stratification

Mammaprint

It was proved that the first genetic test developed by the Netherlands Cancer Institute and American Rosetta Inc., Mammaprint was clinically effective in 2002 (3,4,37,38). Based on the research results of the clinical trial (MINDACT) in 2016, it was recommended by American Society of Clinical Oncology in 2017 (24).

A total of 70-gene signatures were derived from 98 primary breast cancers, in which 34 patients developed distant metastases within 5 years and 44 patients continued to be disease-free after 5 years. On the basis of the 2.5-fold difference between the metastatic group and the

non-metastatic group, the screened genes were available. Patients are classified by calculating the correlation coefficient between a patient's 70-gene expression levels and the average good-prognosis expression profile. If the correlation coefficient exceeds 0.4, the patient is classified as having a good prognosis; if not, the patients are classified as having a poor prognosis (37).

Oncotype Dx

As one of the most popular genetic tests in American, Oncotype Dx has important prognostic value for hormone receptor positive and lymph node negative breast cancer patients. Furthermore, its clinical validity and utility were verified by the NSABP B14 and B20 clinical trials one after another, which was recommended by the National Comprehensive Cancer Network guideline in 2008 (8,25).

Totally, 21 genes were chosen from 250 candidate genes including 16 tumor-associated genes and 5 reference genes, and the calculated recurrence score (RS) ranged from 0 to 100 (39). From its calculation formula, it can be seen that the recurrence score mainly depends on genes involved in ER signaling, proliferation, and HER2 expression. The higher expression level of "favorable" genes [estrogen receptor (ER) group, GSTM1, BAG1] corresponds to a lower RS (because of a negative coefficient in the RS algorithm), whereas the higher expression of "unfavorable" genes (proliferation group, human epidermal growth factor (HER)-2 group, invasion group, and CD68) corresponds to a higher RS (because of a positive coefficient in the RS algorithm) (39). The patients were categorized into 3 risk stratifications based on RS: low (score <18), intermediate (score >18 but <30), and high (score >30) (8,40).

BCI

BCI is the combination of the two-gene ratio HOXB13:IL17BR (H/I) and the five-gene molecular grade index, ranging from 0 to10 (41). Two models were used to calculate BCI including BCI models-cubic (BCI-C) and linear (BCI-L), and the former is superior to the latter based on the study (22). In the BCI-L model, BCI stratifies early-stage estrogen receptor-positive and lymph node-negative breast cancer patients into three risk groups: low (score <5.0825), intermediate (score >5.0825 but <6.5025), and high (score >6.5025) (22). Another method of classification considers different combinations of H/I and molecular

grade index (MGI): low MGI alone represents a low risk, the combination of low H/I and high MGI refers to an intermediate risk and the combination of high H/I and high MGI means a high risk (20).

PAM50

PAM50 was originally designed for simple identification of molecular subtypes of breast cancer, which reflects significant biological information such as hormone and HER2 signaling pathway, proliferation, and markers of basic phenotype (42). Later, it was developed to evaluate the relapse risk and was approved in 2013 by the US Food and Drug Administration (US FDA). PAM50 contains 50 functional genes and 8 targeted genes, with the calculated result of the risk of recurrence(ROR) (14). According to different factors, ROR is classified into 4 types including ROR combined with subtypes (ROR-S), ROR combined with subtypes and proliferation (ROR-P), ROR combined with subtypes and tumor size (ROR-T), and ROR combined with subtypes, proliferation, tumor size (ROR-PT) (43). Despite these types have different classification values, they all divide the recurrence risk into 3 groups. [ROR-S (<24; 24–53; >53), ROR-P (<12; 12–53; >53), ROR-T (<29; 29–65; >65), PAM50 ROR-PT (<18; 18–65; >65)].

Endopredict

The genomic score of Endopredict (EP) considers 8 functional genes and 3 reference genes (44), and among these genes, the expression level of proliferation genes and the estrogen receptor 1 (ESR1) signaling/differentiation—associated genes can predict not only recent relapse but also late recurrence additionally (45). EPscore (0 to 15) divided the risk into two groups including low risk (EP <5) and high risk (EP >5). EPclin is the combination of EP score and two clinical factors (nodal status and tumor size), and its predictive power exceeds that of EP score alone (46).

Prognosis

Oncotype

The prognostic value of 21-gene test was verified for the first time in NSABP B14 trial in 2004 (8). In the low, medium and high-risk lymph node negative and hormone receptor positive breast cancer, the 10-year distant recurrence rates were 6.8%, 14.3% and 30.5% respectively, which indicates that the recurrence score has a significant predictive ability independent of tumor size and age. NSABP B14 supported the application of Oncotype Dx in tumors without lymph node metastasis, and several subsequent studies later have proved that RS could also accurately predict the relapse of lymph node positive patients. The ECOG2107 (9) and TransATAC (10) trials both recruited patients with hormone receptor positive and lymph node positive breast cancer, thus providing robust evidence that RS was significantly associated with relapse regardless of lymph node status. For hormone receptor positive breast cancer patients, 21 gene detection is a more accurate predictor of recurrence than the standard clinical features, and can be used to select low risk patients for simplified chemotherapy regimens. Besides, the phase III trial, SWOG8814 (11) was designed especially for postmenopausal women with node positive and estrogen receptor positive breast cancer and confirmed the prognostic value of Oncotype Dx. Based on the results of the SWOG8814 study, the NCCN guidelines have already been adjusted to include the assay in patients with 1–3 positive lymph nodes to guide the clinical decision. The NSABP B28 further indicated that RS maintained significant prognostic impacts on ER-positive and node-positive patients treated with adjuvant chemotherapy plus tamoxifen (13). The trials mentioned above are all retrospective trials and few studies can provide prospective data to validate the predictive value of Oncotype Dx. In the WGS plan B trial (12,47), RS was prospectively used to define a subset of patients who received only endocrine therapy, which indicated that patients with enhanced clinical risk and omitted chemotherapy on the basis of RS ≤11 had excellent 3-year survival. In addition, the RxPONDER trials is still ongoing to evaluate the 21-gene profile in node positive early breast cancer (48).

Mammaprint

The initial discovery study was performed by van 't Veer in 2002 with 78 patients selected specifically to explore the gene expression signatures of distant metastases (3). Meanwhile, in this study, an additional independent set was selected to validate the prognostic classification. Since the initial discovery cohort of 78 patients was included in the calculation, it was not regarded as a true validation. Therefore, the truly initial validation study of 70-gene assay was conducted by van de Vijver in the same year, which recruited a series of 295 stage I or II breast cancer consecutive patients who were younger than 53 years old (4).

Among the cohort, the mean overall 10-year survival rates were 54.6% and 94.5%, respectively. The probability of remaining free of distant metastases at 10 years was 50.6% in patients with a poor-prognosis signature and 85.2% in patients with a good-prognosis signature. The estimated hazard ratio for distant metastases in patients with poor-prognosis signatures was 5.1, compared with patients with the good-prognosis signature. This validation study demonstrated that Mammaprint was a powerful predictor of the disease outcome. Subsequently, the TRANSBIG (5) and RASTER (6) studies both achieved similar conclusions, which proved that the 70-gene assay provided substantial prognostic value in patients with early breast cancer. All of the aforementioned studies are retrospective and based on data from patients receiving a variable amount of adjuvant therapy. The patients were not the respective of the modern breast cancer patient populations, in which almost all estrogen receptor positive patients received endocrine therapy alone. Therefore, the prospective community-based study (RASTER) (6) was conducted to evaluate the feasibility of Mammaprint in community hospitals and it was the first study aimed to prospectively evaluate the performance of 70-gene signatures. It was found that the 5-year follow-up data confirmed the additional prognostic value of 70-gene signatures for clinicopathological factors could be used in AOL risk assessment.

Endopredict

In 2011, the ABCSG-6/8 study confirmed the possibility of EP in predicting the distant recurrence of estrogen receptor positive and HER2 negative breast cancer patients who received adjuvant endocrine therapy (17). Both trials displayed significant differences in distant recurrence rates between low-risk and high-risk patients classified by EP. In addition, EPclin score was also verified in the ABCSG-6 and ABCSG-8 cohorts, and it was confirmed that EPclin is a continuous predictor of distant recurrence at 5 or 10 years. GEICAM/9906 trial conducted prospective and retrospective clinical verification of EP in patients with estrogen receptor positive and HER2 negative breast cancer patients with positive lymph nodes (18). This study for the first time proved that EP was an independent prognostic parameter of metastasis-free survival (MFS) and overall survival (OS) in lymph node positive, estrogen receptor positive and HER2 negative breast cancer patients.

PAM50

The 50-gene predictor was validated in 2009, and 761 patients who accepted no systemic therapy were evaluated for prognosis, and 133 patients were evaluated for prediction of pathologic complete response to the Taxane and Anthracycline regimen (14). Diagnosis by intrinsic subtype adds significant prognostic and predictive information to standard parameters for breast cancer patients. Both ABSCG8 and transATAC trials (16) chose 2,137 postmenopausal women with hormone receptor positive early breast cancer to predict distant recurrence after 5 years of follow-up, demonstrating that the ROR score added clinically significant prognostic information to the Clinical Treatment Score in all subgroups in the late follow-up period. In 2020, a study recruiting 1,723 breast cancer survivors not only validated the prognostic value of PAM50, but also found that incorporating the 13-gene hypoxia signature into the existing PAM50 risk assessment tool may refine risk stratification and further clarify treatment for breast cancer (49).

BCI

In 2008, Ma and coworkers not only developed a simple gene expression index for tumor grade (molecular grade index or MGI), but also tried to find out whether MGI and previously described HOXB13:IL17BR index together provide improved prognostic information (20). They selected five cell cycle-related genes to build MGI and evaluated MGI in two publicly available microarray data sets including a total of 410 patients. Meanwhile, two additional cohorts (n=323) were used for MGI to validate its prognostic utility, and examine its interaction with HOXB13:IL17BR. As a result, the study proved that the combination of MGI and HOXB13:IL17BR outperformed either alone and identified a subgroup (approximately 30%) of estrogen receptor positive early breast cancer patients with poor outcome despite endocrine therapy. Later, several studies (21-23,50) were performed to validate its prognosis in estrogen receptor positive and lymph node negative breast cancers.

Prediction of treatment benefit

Oncotype Dx

In 2006, Paik and his colleagues performed an NSABP

B20 trial (25) to figure out the relationship between the RS and chemotherapy benefit. A total of 651 patients were enrolled, in which 227 were randomly assigned to tamoxifen alone, and 424 were randomly assigned to tamoxifen plus chemotherapy. The overall benefit of chemotherapy was visible, but when the data was processed by risk category, the benefit was restricted to patients with high RS [RR =0.26 (CI, 0.13 to 0.53)]. Although, patients with intermediate-RS didn't show great benefit, clinically significant benefit cannot be excluded. Subsequently, the SWOG8814 trial (11) also exhibited that Oncotype Dx could predict the significant benefit of chemotherapy in tumors with a high recurrence score. However, it is obvious that patients with high RS derive substantial benefit from systemic therapy, and conversely, patients with low RS don't derive any additional benefit. Most importantly, it is still unclear whether patients with intermediate RS can benefit from systematic therapy. A prospective clinical trial (TAILORx) in 2018 further confirmed that the two groups of patients with RS (11 to 25) were treated with endocrine therapy and chemotherapy plus endocrine therapy respectively. After 9 years, the invasive disease-free survival rate was similar (83.3% and 84.3%), the distant or local invasive disease-free survival (IDFS) was 92.2% and 92.9% and OS was 93.9% and 93.8%, which indicates that the two treatment regimens have similar efficacy in such patients. In this study, whether chemotherapy was used or not, the 9-year distant recurrence rate of women with RS (11 to 25) was about 5%, so it can be concluded that there is little benefit from chemotherapy for patients with RS <26 (26,47). Later, according to the TAILORx secondary analysis (51), the estimated rate of freedom from recurrence in women who received adjuvant chemotherapy regimens plus endocrine therapy, with the RS ranging from 26 to 100, was 93% at 5 years, and the outcome is better than the expected results of endocrine therapy alone in this population.

Mammaprint

The MINDACT trial (24,47) recruited 6,693 lymph node positive women and used prospective evidence to demonstrate the clinical utility of adding Mammaprint to standard clinicopathological criteria to improve the regimens of adjuvant chemotherapy. A total of 1,550 patients (23.2%) were considered to be at high clinical risk and low genomic risk. Among patients who did not receive chemotherapy, the 5-year survival rate without distant metastasis was 94.7% (95% CI: 92.5–96.2). The absolute

difference in survival between these patients and those receiving chemotherapy was 1.5%, and the survival without chemotherapy was lower. In the subgroup of estrogen receptor positive patients, HER2 negative, lymph node negative or lymph node positive diseases, the survival rate without distant metastasis was similar which suggests that in the early breast cancer women with high clinical risk and low genome recurrence risk, the 5-year survival rate of distant metastasis without chemotherapy based on the 70-gene signature is 1.5% lower than that of chemotherapy. In light of these findings, about 46% of patients with high risk of breast cancer may not need chemotherapy. At the same time, the American Society of Clinical Oncology and the European tumor markers group also recommend the use of Mammaprint.

The phase 3 randomized the MINDACT trial with exploratory analysis by age has been conducted with a more mature follow-up, nearly 9 years. For 6,693 early invasive breast cancer patients, 70-gene test displayed complete ability to identify a subgroup of women with high clinical risk, and patients with lower genome risk. When receiving endocrine therapy alone, this subgroup had excellent distant metastasis survival rate, and the benefit of adding chemotherapy to endocrine therapy remained little (2.6%).

Endopredict

The ABCSG 34 trial (28) was conducted in 2020 to assess the ability of the 12-gene molecular score to predict response to Neoadjuvant chemotherapy and endocrine therapy. In this study, the EP score predicted residual cancer burden after treatment with neoadjuvant therapies for patients with hormone receptor positive, HER-2 negative early breast cancer. Tumors with low scores were unlikely to benefit from Neoadjuvant chemotherapy, whereas a high score predicted resistance to neoadjuvant endocrine therapy. This additional biological information can help with personalized treatment selection in daily practice and build a strong rationale to use EndoPredict in the neoadjuvant setting. In the same year, the UCBG 2-14 trial was performed to discuss the clinical utility for patients with intermediate risk and the result showed that EPclin was clinically useful in deciding whether or not to administer adjuvant chemotherapy in patients with intermediate risk (29).

PAM50

Recently, a comprehensive nationwide Danish cohort

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consisting of postmenopausal women with hormone receptor positive early breast cancer treated with 5-years endocrine therapy alone was examined in the DBCG77B trial (27). This study showed that PAM50 could reliably identify node negative patients and a significant proportion of lymph node positive patients who can be spared treatment with adjuvant chemotherapy in the real-world setting.

BCI

Extending the duration of adjuvant endocrine therapy reduces the risk of recurrence in a subset of women with hormone receptor positive early breast cancer, thus the aTTOM trial (30) was performed to evaluate for its ability to predict benefit from extended endocrine therapy. In this study, patients with high BCI derived a significant benefit from extended tamoxifen treatment with 10.2% of absolute risk reduction, while patients with low BCI showed no significant benefit from extended endocrine therapy. Further, similar conclusion was seen in the aTTOM trial, demonstrating that BCI predicted preferential benefit from extended endocrine therapy and identified patients with improved outcomes from completing 10 years of adjuvant endocrine therapy (31).

Discussion

With the development of precision medicine, the genetic test plays an important role in clinical decision-making. Only one gene can't decide prognosis simply, so multiple genes are needed to address this problem. In general, this technology aims to identify molecular subtypes, evaluate prognosis, predict treatment outcome and make clinical decisions.

Almost all genetic tests share the same developing procedures, and experience the selection of associated genes, the analysis of clinical validity and utility, and marketing approval. Their techniques and principles of them were identical and they should follow the same marketing rules. Their technology and principle are the same, their utility must be verified by a large number of clinical trials, and their clinical application must be recommended by professional guidelines (32). Among them, it is worth noting that the popularity of Mammaprint is limited because it requires fresh frozen tissue and needs to be sent to the central laboratory. Fortunately, the new

version of Mammaprint has been applied to FFPE, and a new prognostic model is under construction.

In this updated version of the American Joint Committee on Cancer (AJCC) eighth edition, the significance of multigene assays for breast cancer was first emphasized. The above five multigene testing techniques were recommended, and Oncotype Dx was recommended as class I evidence, which confirmed the importance of multi gene analysis in the diagnosis and treatment of breast cancer. Although breast cancer multigene testing technology has been used in clinical practice for many years, many studies have proved that it has significant value in providing prognostic information and guiding treatment. On the contrary, specific clinical applications suggest different opinions on different expert consensus and guidelines (*Table 3*). For example, in the latest NCCN guidelines, hormone positive and HER2 negative patients with 1–3 lymph nodes considered Oncotype Dx to analyze the recurrence score to guide the addition of chemotherapy to standard hormone therapy, while ASCO guidelines only recommended the addition of chemotherapy to patients with negative lymph nodes.

Compared with classical clinicopathological characteristics, gene signatures may provide more predictive value, while there are still a large number of problems to be addressed. First of all, this technology is very complex. The Samples for different genetic tests have their own standards, and most samples need to be transported to the central laboratory. Secondly, the function of genes contained in gene expression profiles and their effect on tumor characteristics are still unclear. Meanwhile, the epidemiological characteristics of the population in different trials are different, which may lead to different testing results. Thirdly, most of the patients enrolled in these clinical trials have accepted systemic therapy before, so it is hard to distinguish the therapeutic effect from the biological behaviors of tumors. Fourthly, the majority of clinical trials is retrospective studies, and lack prospective proofs to support the genetic test. Lastly, high costs make genetic tests difficult to be accepted.

Conclusions

In conclusion, gene expression profiles are the supplement to immunohistochemical methods, which means that we can choose genetic tests based on classical clinicopathological characteristics, but we cannot skip them. It is still necessary

Table 3 Different expert guidelines recommend the use of multigenic assays

Multigenetic assay	ASCO	NCCN	St. Gallen	EGTM
Oncotype Dx	pN0	pN0; pN1 (1–3 positive nodes)	pN0; pN1 (1–3 positive nodes)	pN0; pN1 (1–3 positive nodes)
Mammaprint	Not determined	Not determined	pN0; pN1 (1–3 positive nodes)	pN0; pN1 (1–3 positive nodes)
Endopredict	pN0	Not determined	pN0; pN1 (1–3 positive nodes)	pN0; pN1 (1–3 positive nodes)
PAM50	pN0	Not determined	pN0; pN1 (1–3 positive nodes)	pN0; pN1 (1–3 positive nodes)
BCI	pN0	Not determined	pN0; pN1 (1–3 positive nodes)	pN0

ASCO, American Society of Clinical Oncology; NCCN, National Comprehensive Cancer Network; EGTM, European Organization Tumor markers; PAM50, mRNA expression of 50 genes; BCI, breast cancer index.

to explore how to select optimal genetic tests, how to choose target population, and how to incorporate genetic tests into clinical decision making more standardly.

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-1920/rc

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-1920/coif). Both authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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