

## Application of multigene panel detection in breast cancer

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### Abstract

Precision medicine will be the direction of future medical development, especially in cancer diagnosis and treatment. With the deepening of breast cancer-related research, new factors related to diagnosis, treatment and prognosis are constantly being discovered. Researchers combine different factors to form a multigene panel testing, guiding clinicians' decision-making. The application scope of multigene panel detection is constantly expanding. At present, it has been tried in the prognosis evaluation of lymph node-positive and human epidermal growth factor receptor 2-positive breast cancer patients and the early screening of breast cancer. With continuous technological advancement, there will be broader application prospects in the future. The current narrative review was planned to evaluate the recent advances in applying multigene panel testing in breast cancer cases.

**Keywords:** Multigene panel testing, Breast cancer, Oncotype Dx, MammaPrint, Endopredict, PAM50, BCI, CancerSeek.

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### Introduction

Breast cancer (BC) surpassed lung cancer in 2020, becoming cancer with the highest incidence in the world (11.7% vs. 11.4%).<sup>1</sup> The incidence of BC is positively correlated with the national economic level; in developed countries, it is much higher than in moderately developed countries. However, most BC deaths occur in middle-income countries because most women in those countries cannot get regular health checks or receive regular treatment.<sup>2</sup> Most BCs can be cured by surgical removal before they metastasise.<sup>3</sup> Even when metastases occur, early detection of metastases combined with appropriate drug treatment can lead to better outcomes. This phenomenon shows the importance of early diagnosis and long-term monitoring in BC treatment. The worldwide recommended screening method for BC is mammography, but its sensitivity and specificity are affected by the density

of breast tissue.<sup>4</sup> The preoperative diagnosis of BC usually relies on an image-guided percutaneous biopsy, but BC is a heterogeneous disease with morphological, molecular and clinical diversity<sup>5</sup> and image-guided percutaneous biopsy removes only part of the tissue and ignores tumour heterogeneity.

In clinical treatment, doctors perform different treatments according to the stage and molecular type of BC. However, according to the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis, traditional clinicopathological factors, such as tumour size, lymph node (LN) staging, age, and hormone receptor (HR) status cannot predict the efficacy of adjuvant chemotherapy. There is evidence that chemotherapy has lower benefits for patients with Luminal A.<sup>6</sup> Some studies have shown that HR-positive, human epidermal growth factor receptor 2 (HER2)-negative BC patients with tumour stage 1 (T1) and node stage 0 (N0), if they do not receive chemotherapy, have a long-term recurrence rate of only 13%.<sup>7</sup> These pieces of evidence prove that although some factors have a clear prognostic effect, routine factors alone may not be enough to determine patients whose absolute risk of recurrence (ROR) is low.

The application of multigene panel testing (MPT) provides a solution to these problems. MPT detects and analyses different combinations of cancer-related genes and proteins to achieve a diagnosis, prognosis, and for other purposes. The American Society of Clinical Oncology (ASCO) approved Oncotype DX<sup>®</sup> and MammaPrint<sup>®</sup> in 2007 and 2017, respectively, to guide adjuvant treatment decisions for early-stage invasive BC.<sup>8</sup>

### 21-gene recurrence score (RS) assay (Oncotype Dx)

At present, the most widely available MPT for BC is the 21-gene recurrence score (RS) assay (Oncotype Dx<sup>™</sup>), which has a level I clinical evidence.<sup>9</sup> The assay is a method to detect the expression level of 16 cancer-related genes quantitatively and 5 reference genes in the ribonucleic acid (RNA) isolated from formalin-fixed paraffin-embedded (FFPE) tissue of node-negative, tamoxifen-treated HR-positive/HER2-negative BC patients, and then convert the gene expression into recurrence risk score by calculating formula and provide guidance for prognosis prediction and treatment of BC patients according to the score.

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In a retrospective analysis of breast tissue samples trial, the 21-gene RS assay's inventor, Paik et al., collected 668 stage I or II LN-negative, HR-positive and tamoxifen-treated BC patients.<sup>10</sup> The risk of recurrence was then assessed using the 21-gene RS assay. The RS score in low-, medium- and high-risk groups of 10-year long-term recurrence rate was 6.8%, 14.3% and 30.5% ( $p < 0.001$ ), which proved the effectiveness of RS score as a prognostic tool for BC patients. It can also assess whether patients need chemotherapy and how much they benefit from chemotherapy. A large prospective study confirmed the value of 21-gene RS assay in adjuvant therapy selection for early BC patients. This study enrolled 10,253 female HR-positive, node-negative and HER2-negative BC patients with with the primary tumour's maximum diameter being 1.1~5.0cm. Of the total, 1,626(15.9%) women had an RS of 0-10 and received endocrine therapy alone. The 5-year disease-free survival (DFS) was 93.8%, the distant metastasis-free survival (DMFS) was 99.3%, and the 5-year overall survival (OS) was 98%. Based on this, the researchers suggest that some luminal early BC patients with good genotypes can only be treated with endocrine therapy within 5 years.<sup>11</sup> For these reasons, many authorities recommend it for BC.<sup>5,11,13</sup> Oncotype Dx's conclusion is a numerically linear continuous risk score, which can accurately predict the risk of recurrence for each patient.

However, the Oncotype Dx intermediate-risk group is in the grey area. It is unclear whether this group of patients can receive clear chemotherapy guidance from the test. A large-scale prospective trial aimed at illustrating Oncotype Dx's problem, evaluating whether each group can benefit from adjuvant chemotherapy.<sup>11</sup> However, the researchers wanted to minimise the possibility of inadequate treatment for the subjects, and narrowed the risk definition and set the RS low/medium-risk and medium/high-risk threshold value from the originally designed 18 and 31 to 11 and 26, respectively. Patients with an RS score  $\leq 10$  were classified into the low-risk group, who received endocrine therapy only. Patients with RS score 11-25 were divided into the middle-risk group, and were randomly assigned to endocrine therapy alone or endocrine therapy plus adjuvant chemotherapy. Patients with RS score  $\geq 26$  were placed in the high-risk group, which required endocrine therapy plus adjuvant chemotherapy. According to the latest results of the experiment released in 2019, the expert panel recommended that patients with RS scores  $< 26$  should receive endocrine therapy alone; patients with RS scores 16-25 and aged  $< 50$  may choose chemotherapy; patients with RS score 30 should undergo chemotherapy plus endocrine therapy.<sup>14</sup> Whether adjuvant chemotherapy can improve the prognosis of intermediate-risk patients still needs to wait for longer follow-up results of the ongoing

study. Moreover, whether the grouping threshold's adjustment will affect the external authenticity of its conclusions or lead to bias also remains to be evaluated.

In a recently published study, researchers tracked the prognosis of patients with RS scores ranging 0-25 and with 1-3 positive LNs. A total of 5,083 patients were enrolled, with a median follow-up of 5.3 years, and 99 subjects withdrew midway. The final result showed that for premenopausal BC patients with 1-3 positive LNs, if the RS score is 0-25, adjuvant chemotherapy can be omitted without affecting the patient's progression-free survival (PFS) and DMFS.<sup>15</sup>

### 70-gene prognostic signature (MammaPrint)

Van't Veer et al. of the Netherlands Cancer Institute published a retrospective study in 2002.<sup>16</sup> The study enrolled 78 HR-positive, HER2-negative BC patients with age  $< 55$  years, no LN metastasis, and tumour diameter  $< 5$ cm. The complementary deoxyribonucleic acid (cDNA) microarray technology containing 25,000 genes was used to analyse the RNA of the patient's fresh frozen tissue which was calculated by unsupervised hierarchical clustering method. A set of 70 genes most relevant to prognosis were screened step by step. These 70 target genes contained genes related to cell cycle regulation, proliferation, angiogenesis, invasion, metastasis, signal transduction, and other cancer biological characteristics, constituting the MammaPrint detection system. This MPT method is the oldest among several widely-recognised methods. Subsequently, the research team expanded the sample size to verify the prognosis and prediction capabilities of MammaPrint. With the same enrollment conditions, 295 patients with invasive BC were added. According to the MammaPrint test, 40% of patients had a good prognosis group, and 60% had a poor prognosis. The average 10-year survival rates of the poor prognosis group and the good prognosis groups, respectively, were  $54.6\% \pm 4.4\%$  and  $94.5\% \pm 2.6\%$  ( $p < 0.001$ ), and the 10-year survival rate without distant metastasis was  $50.6\% \pm 4.5\%$  and  $85.2\% \pm 4.3\%$  ( $p < 0.001$ ). The risk of distant metastasis in the poor prognosis group compared with the good prognosis group was 5:1. Multivariate Cox regression analysis showed that MammaPrint is an independent risk factor that effectively predicts BC's outcome. The validation study showed that MammaPrint had an important value in predicting the prognosis of BC.

In a study, Buyse et al. evaluated the prognosis of 302 patients with BC using MammaPrint detection and clinicopathological analysis (Adjuvant! Online (AOL)), with a median follow-up of 13.6 years. In 87(29%) cases, the results were inconsistent between the two evaluation

systems. There were 59(68%) patients whose ROR was high-risk in AOL and low-risk in MammaPrint, the 10-year OS rate was 89%. Besides, 28(32%) cases had a low risk on AOL and high risk on MammaPrint, and their 10-year OS rate was 69%.<sup>17</sup> Based on the clinical verification data of the study, the United States Food and Drug Administration (FDA) approved the MammaPrint product to be launched in 2007.

A trial compared MammaPrint and AOL results, screened high-risk patients with AOL, and used MammaPrint genetic risk to guide chemotherapy decisions, providing clinical benefits and avoiding over-treatment of some patients.

The application of MammaPrint also has certain limitations. Although the detection system can predict the possible benefit of chemotherapy in risk groups, it cannot predict its sensitivity to specific chemotherapy drugs. The research on the benefit of chemotherapy only focusses on more classic chemotherapy regimens even though some early chemotherapy regimens have been replaced by newer ones due to difference in side effects. The applicability of MammaPrint has yet to be confirmed, and whether MammaPrint can be used for prognostic risk prediction for patients after chemotherapy is also an open question.

### 12-gene molecular score (Endopredict)

In 2011, Martin et al. found that none of the MPT methods recommended by the guidelines predicted HR-positive, HER2-negative BC recurrence better than a combination of HR, HER2, and Ki67 (Ki67) assayed by immunohistochemistry (IHC).<sup>18</sup> They sequenced tissue samples' genes from 946 HR+, HER2- BC patients, then selected 12 tumour-related and reference genes through a top-down approach to calculate a risk score, and called it Endopredict (EP), which, combined with nodal status and tumour size, led to a comprehensive risk score; EPclin. The team then validated the two risk scores in two large phase-III clinical trials. Finally, multivariate analysis showed that EP could be an independent predictor of recurrence as well as 10-year DMFS. These results demonstrate that EP is a better predictor of BC recurrence than conventional clinicopathological factors. Endopredict can also guide the selection of preoperative neoadjuvant chemotherapy (NaCT).

Dubsky et al. selected 217 HR-positive, HER2-negative BC patients from a randomised phase-II trial to perform the EP test.<sup>19</sup> Of these patients, 134 received NaCT, and 83 received neoadjuvant endocrine therapy (NET). Low-risk NaCT-treated and high-risk NET-treated tumours responded poorly, while in the other patients, EP showed a good predictive effect for NaCT and NET. Buns et al. compared EP, EPclin, and Oncotype DX's value in predicting

the risk of BC metastasis over 10 years.<sup>20</sup> The study showed that for BC patients with HR-positive and HER2-negative status, except for the LN-negative group within 5 years, the predictive value of EPclin was higher than that of EP; within 5 years after surgery, EP and Oncotype DX had the same prognostic value; while after 5-10 years, both EP and EPclin had better predictions of distant metastasis than Oncotype DX. EPclin provided more prognostic information than RS.

### PAM50(ProSigna)

In 2009, Parker et al. established a detection model based on 50 BC-related genes and 5 reference gene.<sup>21</sup> First, they analysed the data of 189 prototype samples, and screened out 50 genes that can be used for subtyping the intrinsic subtypes of BC. Then, they used the model to evaluate the prognosis of 761 patients who did not receive systemic treatment, and predicted pathological complete remission (pCR) for 133 patients who received chemotherapy. The results showed that the intrinsic subtyping of BC with PAM50 had significance in prognostic prediction ( $p < 0.0001$ ), and the negative prediction rate of pCR after NaCT was 97%.

Since then, some researchers have further developed the model. The PAM50-based Prosigna ROR score combines these genes with clinical information. After analysing these prognostic factors, they are grouped into a single number, and the score ranges 0-100. It can be used to predict the risk of distant recurrence within 10 years of standard treatment for HR-positive, HER2-negative BC patients with tumour diameter  $\leq 5$ cm and positive LNs  $\leq 3$  after early menopause. A study was conducted in this regard with median follow-up of 9.2 years.<sup>22</sup> Chemotherapy is not recommended for the low-risk ROR group. Therefore, ROR can help some patients avoid chemotherapy and its side effects.<sup>23</sup> In addition, research on whether ROR can identify patients who can benefit from endocrine therapy for  $> 5$  years is also ongoing.<sup>24</sup>

Other scholars added other tumour-related factors to improve PAM50's prediction of prognosis. Pu et al. performed PAM50 detection on 1,723 BC samples, obtained 1,253 high-quality readings, and evaluated these patients for a 15-year long-term follow-up. The results showed that the PAM50 subtype was related to BC prognosis after taking into account the tumour stage, grade, and age at diagnosis ( $p < 0.01$ ). The state of menopause at the time of diagnosis did not affect the prognosis. Compared with the Luminal A subtype, the Luminal B subtype had a 60% higher risk ( $p < 0.0001$ ). The addition of a 13-gene hypoxia signature improved prognostication with  $> 40\%$  higher hazard in the highest versus lowest hypoxia group ( $p = 0.04$ ).

However, the disadvantage is that it does not incorporate the expression of immune genes in tumour tissues closely related to the prognosis of BC into the risk assessment system. As such, the risk assessment of transfer needs to be improved.

### Breast Cancer Index (BCI)

US company BioTheranostics invented the Breast Cancer Index (BCI), which measures and reports the expression of seven BC-related genes, including the 5-gene molecular grade index and HOXB13/IL17BR (H/I), and divides the recurrence risk of HR-positive BC patients into high-risk, intermediate-risk and low-risk categories.<sup>25</sup> This helps the clinicians decide whether or not to extend the patient's 5-year endocrine therapy to 10 years.

According to the latest results of a multi-centre trial, researchers used BCI to evaluate 583 HR-positive and N-positive BC patients, and 49% classified as BCI high-risk derived a significant benefit from 10 versus 5 years of tamoxifen treatment, while BCI low-risk patients showed no significant benefit from extended endocrine therapy.<sup>26</sup>

BCI can also be used as an independent prognostic factor for invasive lobular carcinoma of the breast. Nunes et al. analysed 307 patients with invasive lobular carcinoma, and showed significant differences in distant recurrence (DR) based on BCI risk classification over the preceding 10 years.<sup>27</sup> The DR rates of BCI low-risk and intermediate-risk patients were similar (7.6% and 8.0%, respectively), while the DR rate of BCI high-risk patients was 27%. BCI was found to be an important independent prognostic factor for 10-year DR ( $p=0.0001$ ) in early-stage ( $p=0.0042$ ) cases and in late-stage cases ( $p=0.0224$ ). In multivariate analysis, BCI was found to be the only statistically significant prognostic factor for DR ( $p=0.0150$ ).

### Cancer SEEK

The most promising role of MPT is to screen for cancer in healthy, asymptomatic populations and detect cancer earlier. Early diagnosis of BC is important because early detection of tumours remains the most effective way to reduce mortality. The window period between the appearance of cancer cells and the onset of metastasis is a golden period for detecting and diagnosing cancer. CancerSEEK is a non-invasive multigene panel for early screening for eight cancers, including BC. Researchers first selected 61 amplicons and 41 cancer-related proteins from 16 cancer-related genes from data published on the internet, and then further screened out 8 of 41 cancer-related proteins through further verification. Eight kinds of proteins and 61 amplicons make up CancerSEEK (Table).<sup>28</sup> After that, they performed CancerSEEK tests on 1,005 confirmed cancer patients and 812 healthy people. The

highest sensitivity was 98% for ovarian cancer, the lowest was 33% for BC, the median sensitivity was 70%, and the specificity was >99%. Besides, when using the two most likely cancers as effective values, BC's prediction accuracy reached 83%. However, most of the tumour patients enrolled in the study had symptomatic cancer, and the detection rate of CancerSEEK may be lower for asymptomatic patients. Moreover, their control groups were all healthy people, but in practice, chronic diseases and inflammation may lead to higher false positives.<sup>29</sup> Another large prospective interventional study was conducted to verify the effectiveness of the liquid biopsy method in the general population. It recruited 1,0006 women aged 65-75 years with no history of cancer. CancerSEEK was used for the first test. If it was positive, further tests and expert demonstrations were carried out. After the above three steps, if it was still positive, positron emission tomography-computed tomography (PET-CT) was used.<sup>30</sup> In the 12-month study, a total of 96 cases of cancer were diagnosed, of which CancerSEEK found 26(17.1%), and the sensitivity was 52.1% after combining with standard screening methods. Its specificity was 98.9% and 99.6% when combined with standard screening methods. The study increased diagnostic specificity to 99% with a two-step blood test. Patients who tested positive were evaluated by a professional committee for further PET-CT examination. Thus, the test would not affect patients undergoing standard screening, demonstrating its safety and effectiveness.

### Other multigene panels

In addition to the multigene panels mentioned above, there are evaluation tools based on pathological characteristics, such as clinical treatment score at 5 years (CTS5), and immunohistochemical-4 (IHC4) index scores. IHC4 uses a mathematical formula to measure the semi-quantitative expression values of oestrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 detected by IHC, and combines these values into a single risk score. The recently announced CTS5 is the only predictive model designed to assess the long-term risk of distant recurrence in postmenopausal patients. Its formula includes the patient's age of onset, tumour grade, tumour size, and the number of positive LNs.<sup>31</sup>

In 2018, a retrospective study compared Oncotype, ROR, BCI, EPclin, CTS5 and IHC4<sup>32</sup> with respect to predictive value of distant recurrence after 0-10 years and 5-10 years. The 6 tools performed similarly in predicting recurrence from 0-10 years, but PAM50, BCI and EPclin performed better in predicting long-term recurrence at 5-10 years. Although the three tools are not specifically used to assess the risk of long-term recurrence, they all showed good predictive value.



**Table:** Overview of the four kinds of multigene panel testing.

	First published in	Range of application	Sample required for testing	Test content	Decision criteria	Related research
<b>Oncotype Dx</b>	Paik S, et al. <sup>10</sup> J Clin Oncol. 2006 Aug 10;24 (23): 3726-34.	pT1-2,ER/PR+, Her2-,pN0 Invasive breast cancer	FFPE tissue	16 cancer related genes and 5 reference genes	Low risk: RS<18: The benefit of chemotherapy is low, so endocrine therapy alone is recommended Intermediate risk: 18≤RS<31: No specific treatment is recommended High risk:RS≥31: Higher chemotherapy benefit	NSABP B-14 NSABP B-20 NSABP B-28 TAILORx
<b>MammaPrint</b>	van 't Veer LJ, et al. <sup>16</sup> Nature 2002;415:530–6.	pT1-2,ER/PR+, Her2-,pN0 age<55 years Invasive breast cancer	Frozen tumour tissue	70 cancer related genes	Low risk: Endocrine therapy alone High risk: Higher chemotherapy benefit	Mindact TRANSBIG
<b>Endopredic</b>	Filipits M, et al. <sup>18</sup> Clinical cancer research : an official journal of the American Association for Cancer Research 2011;17:6012-20	pT1-2,ER/PR+, Her2-,pN0 Invasive breast cancer	FFPE tissue	8 cancer related genes and 4 reference genes	Low risk: The 10-year DR rate was 4% High risk: The 10-year DR rate was 28%	ABCSG06 ABCSG08
<b>PAM50(ROR)*</b>	Parker JS, et al. <sup>21</sup> J Clin oncol. 2009;27(8):1160-7	Intrinsic subtypes: Invasive breast cancer ROR: pT1-2, positive lymph nodes≤3, ER/PR+, Her2-, Invasive breast cancer	Frozen tumour tissue or FFPE tissue	50 cancer related genes and 5 reference gene	Node-positive: Low ROR VS. High ROR: The 10-year DR rate was 3.5% vs.22.1% Node-negative: Low ROR VS. High ROR:The 10-year DR rate was 5% vs.17.8%	TransATAC ABCSG-8
<b>BCI</b>	Ma XJ, et al. <sup>25</sup> Clin Cancer Res. 2008;14(9):2601-8	pT1-2,ER/PR+ Invasive breast cancer	FFPE tissue	5-gene molecular grade index and HOXB13/IL17BR (H/I)	Low-risk:10-year DMFS is 98%(96-100%) Intermediate risk:10-year DMFS is 87%(77-99%) High-risk:10-year DMFS is 60%(47-78%)	Trans-aTTom
<b>CancerSEEK</b>	Cohen JD, et al. <sup>28</sup> Science 2018;359:926-3	Breast cancer, Ovarian cancer, Liver cancer, Gastric cancer, Pancreas cancer, Esopsagus cancer , Colorectum cancer, Lung cancer	Blood	16 cancer related genes and 8 reference protrins	Any positive test results are recommended for further imaging examination	DETECT-A

\*The ROR score is also divided into 3 groups, but the intermediate risk group did not give the corresponding recurrence rate and no treatment recommendations, so it is not listed in the table. ROR: Risk of recurrence, FFPE: Formalin-fixed paraffin-embedded, DMFS: Distant metastasis-free survival, ER: Oestrogen receptor, PR: Progesterone receptor.

The development and application of tumour genomics have brought BC diagnosis and treatment to a new era. Revealing the essence of tumours from the biological characteristics and achieving individualised and precise treatment of tumours is likely to be the direction of tumour treatment. In some head-to-head studies, MPT was not consistent in predicting the prognosis of BC patients<sup>33</sup> but large, prospective studies are still going on. One set of researchers believed that RS mainly reflects characteristics related to oestrogen, while EP, BCI and PAM50 mainly reflect characteristics related to proliferation, which can also explain the difference in prognostic prediction performance.<sup>34</sup>

The continuous development of liquid biopsy in recent years has brought more promising prospects for MPT for BC patients.<sup>35</sup>

## Conclusion

Compared to the traditional clinicopathological factors, multigene detection tools may provide more accurate diagnosis and prognostic prediction, and provide a reference for some patients to choose treatment options, which should become an important breakthrough direction for precise future treatment of BC.

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