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*Review Article*

# Application and challenge of HER2DX genomic assay in HER2+ breast cancer treatment

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**Abstract:** HER2-positive breast cancer is highly aggressive, with a significant risk of recurrence and metastasis, leading to a poor prognosis. While most early-stage HER2-positive breast cancer patients benefit from combining trastuzumab monoclonal antibody with chemotherapy, the therapeutic response to various drug combinations varies across the HER2+ patient population. Therefore, predicting the prognosis and treatment response of HER2+ breast cancer patients to specific regimens is crucial for selecting appropriate precision individualized therapies. HER2DX is the first genomic tool designed to guide the treatment of HER2+ breast cancer patients. The three scores provided by HER2DX inform the entire treatment process, including predicting survival outcomes, recurrence, metastasis, and treatment responses like Pathological Complete Response Rate (pCR). It offers recommendations on follow-up intervals, treatment plans, and the duration of drug therapy. This review examines the literature and analyzes studies applying HER2DX to guide the comprehensive treatment and predict prognosis in HER2+ breast cancer patients, aiming to promote the widespread use of HER2DX in individualized treatment.

**Keywords:** Breast cancer, HER2, HER2DX, pCR, Outcomes

## Introduction

The latest data from the International Agency for Research on Cancer (IARC)'s Global Cancer Observatory indicates that 2.3 million people were diagnosed with breast cancer in 2022, making it the second most common cancer worldwide. About 670,000 people died from breast cancer, ranking it fourth in cancer mortality rates [1]. Among women, breast cancer, lung cancer, and colorectal cancer are the most common cancer types, accounting for 51% of all new cancer cases in women, with breast cancer alone constituting 32% [2]. In the majority of countries (157 out of 185), breast cancer is the most common malignant tumor in women [3]. In China, there were approximately 2.29 million new cancer cases among women in 2022, with 357,161 new cases of breast cancer, making it the second most common cancer among Chinese women [1], underscoring the significance of breast cancer as a major health concern.

Breast cancer is categorized into four molecular subtypes based on gene expression profiles and biomarkers: Luminal A, Luminal B, HER2-positive (HER2+), and triple-negative breast cancer (TNBC), with HER2+ breast cancer accounting for 15%-20% of all cases [4]. The human epidermal growth factor receptor-2 (HER2/ERBB2) is a proto-oncogenes associated with inhibiting apoptosis and promoting cell proliferation, which enhances tumor invasiveness and promotes angiogenesis and lymphangiogenesis [5, 6]. The HER2 protein, encoded by the ERBB2 gene, is a transmembrane protein with tyrosine kinase activity and is part of the EGFR family [5, 7]. This family includes HER1, HER2, HER3, and HER4, all characterized by an extracellular domain, an α-helical transmembrane region, and an intracellular tyrosine kinase domain [8-10]. The HER2 receptor does not directly bind to any known ligand; its extracellular domain remains in an "open" conformation [11]. It functions in signal transduction either by forming homodimers or heterodimers with other

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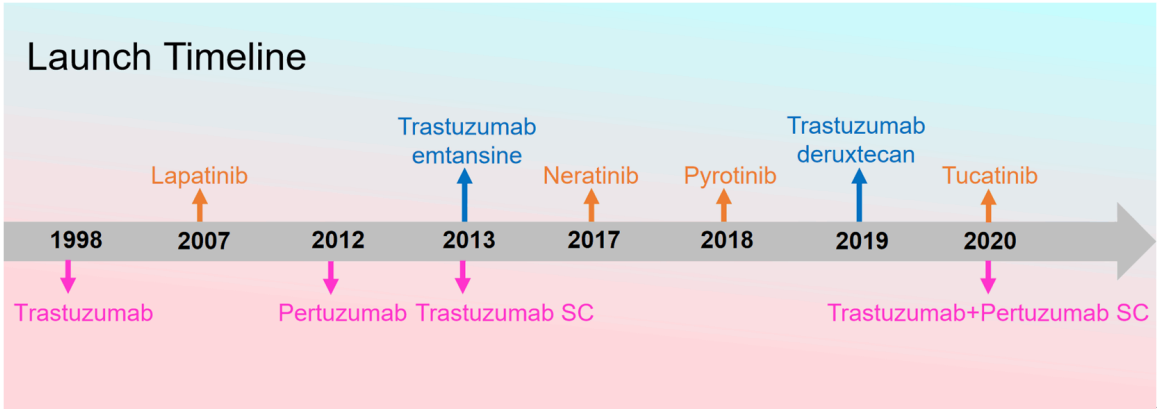


**Figure 1.** Global launch timeline of anti-HER2 targeted drugs.

HER family members upon ligand binding to their extracellular domains, predominantly through heterodimerization [12, 13]. Upon dimerization, HER2 undergoes a conformational change, activating its intracellular tyrosine kinase activity, which initiates downstream signaling pathways, promoting cell proliferation through the RAS-ERK pathway and inhibiting cell death via the PI3K-Akt-mTOR pathway [14-17].

HER2+ breast cancer is highly aggressive, with patients often experiencing early recurrence and metastasis, leading to poor prognosis [18-20]. Initially, systemic chemotherapy was the primary treatment for HER2+ breast cancer patients, but it demonstrated unsatisfactory clinical efficacy [21]. The advent of anti-HER2 targeted therapy drugs, characterized by their specificity, effectiveness, and fewer adverse reactions, has significantly improved the prognosis for patients with HER2+ breast cancer and has become the main treatment approach [22, 23]. Currently, anti-HER2 therapy drugs are mainly categorized into three groups: monoclonal antibodies (such as trastuzumab, pertuzumab, margetuximab, and ado-trastuzumab emtansine), small molecule tyrosine kinase inhibitors (TKIs) (such as pyrotinib, lapatinib, neratinib, and tucatinib), and antibody-drug conjugates (ADCs) (such as T-DM1, T-DXd, and RC-48). With the growing clinical demand for diverse anti-HER2 targeted drugs, the development and clinical application of these therapies continue to advance (**Figure 1**).

Large molecule monoclonal antibodies primarily target the extracellular domain of HER2, aiming to block the HER2-mediated signaling pathways. For instance, trastuzumab, a monoclonal antibody, specifically binds to the IV domain of the HER2 extracellular region, accelerating HER2 internalization and degradation, thereby inhibiting downstream signaling and exerting its antitumor activity. It also mediates antibody-dependent cellular cytotoxicity, killing cells that express HER2-related proteins [24-26]. Pertuzumab monoclonal antibody binds to the II domain of the HER2 extracellular region, preventing the formation of heterodimers between HER2 and HER1, HER3, HER4 [27, 28]. Clinical studies such as CLEOPATRA, PUFFIN, NeoSphere, and PEONY have confirmed the significant efficacy of combining trastuzumab and pertuzumab in HER2+ breast cancer patients [29-32]. Small molecule TKIs diffuse through the cell membrane and competitively inhibit the binding of ATP to the ATP-binding site of the intracellular kinase domain of the HER family proteins, thereby blocking tyrosine phosphorylation and the activation of downstream signaling cascades, which suppresses the growth and proliferation of cancer cells [25, 33-36]. Numerous clinical studies have demonstrated the significant therapeutic effects of TKIs in patients with HER2+ breast cancer [37-40]. ADCs consist of an antibody that selectively recognizes the HER2 receptor on the surface of cancer cells, a cytotoxic drug payload capable of killing cancer cells, and a linker connecting the antibody to the payload. Some ADCs also possess cytotoxic effects through their antibody component. These ADCs bind to the HER2 receptors on the tumor cell surface, mediate endocytosis, and are subsequently degraded within the lysosomes of tumor cells, releasing

the active cytotoxic drug that damages DNA or inhibits tumor cell division, leading to tumor cell death [41-43]. Furthermore, certain ADCs like T-DXd and RC-48 exhibit a bystander effect, where cytotoxic drugs released from dying tumor cells exert lethal effects on surrounding non-HER2+ tumor cells [44-47]. Clinical studies such as EMILIA, KATHERINE, DESTINY-Breast01, 02, and 03 have confirmed that ADCs like T-DM1 and T-DXd significantly improve the prognosis of HER2+ breast cancer patients who did not achieve expected results from previous treatment with monoclonal antibodies or TKIs [48-52].

As anti-HER2 targeted therapies have advanced, the prognosis of patients with HER2+ breast cancer has improved. However, clinically, some patients exhibit low response or even resistance to targeted therapies. Therefore, selecting the most suitable targeted therapy regimen for each patient, and deciding between or combining different targeted drugs, is crucial. Breast cancer is a highly heterogeneous disease; each molecular subtype has its unique molecular characteristics and signaling pathways [53]. Previously, decisions to escalate or de-escalate breast cancer systemic therapy were primarily based on traditional parameters such as tumor size, regional lymph node status, hormone receptor (HR) status, tumor-infiltrating lymphocytes (TILs), Ki-67 proliferation index, and pathological type. Among these, TILs can predict chemotherapy efficacy and prognosis for most breast cancers, including TNBC and HER2+ breast cancer subtypes [54, 55]. To more accurately predict the efficacy and prognosis of breast cancer treatment regimens, various prognostic auxiliary models that combine pathology, molecular biology, and biomarkers are currently used, such as Oncotype DX, Prosigna™, Mammaprint, and EndoPredict (**Table 1**). These models assist physicians in making adjuvant treatment decisions and predicting prognoses for early-stage HR+/HER2- breast cancer patients who are premenopausal or postmenopausal, with either negative regional lymph nodes or 1-3 positive regional lymph nodes [56-58]. However, the treatment plans for HER2+ breast cancer patients still primarily rely on traditional parameters and the clinical experience of physicians, lacking genomic tools for aiding decision-making in HER2+ breast cancer. To achieve more precise personalized treatment for HER2+ breast cancer patients, Reveal Genomics has developed the first genomic tool targeted for these patients-HER2DX [59, 60].

HER2DX is a more comprehensive genomic tool compared to earlier ones, which have limitations when used alone in clinical settings. Sestak et al. compared six clinical features (CTS, IHC 4, Oncotype DX, Prosigna, BCI, and EPclin) in the TransATAC cohort to assess their prognostic abilities for distant recurrence over 0-10 years. CTS and EPclin provided the most accurate prognostic information in the 0-10 and 5-10 year periods. In node-negative patients, ROR of Prosigna had the highest prognostic value, while RS of Oncotype DX had the lowest. In node-positive patients, CTS and EPclin were the most prognostic, while the other four features had less predictive value [65, 66]. These findings suggest that genetic testing alone cannot provide comprehensive prognostic information. Effective clinical application of genetic testing requires integration with clinical features. Tools like Oncotype DX, Prosigna, MammaPrint, and EndoPredict were among the first to evaluate prognosis in HR+ breast cancer patients, but multiple studies [65, 67-69] indicate that using these tools alone may introduce bias. Combining them with clinical features is essential for accurate prognosis and appropriate treatment selection.

Unlike these tools, HER2DX is the first designed specifically for HER2+ breast cancer. It combines genetic analysis with clinical features like tumor size and lymph node status. HER2DX not only predicts clinical prognosis but also treatment response to various therapies, making it well-suited for precise personalized treatment in clinical practice. HER2DX integrates genomic data with clinical features to generate three scores: the HER2DX Risk Score, the HER2DX pCR Score, and the HER2DX ERBB2 Expression Score. These scores help predict survival outcomes and the risks of recurrence and metastasis in HER2+ breast cancer patients, guiding treatment strategies. This approach reduces the risks of disease progression due to under-treatment and mitigates economic burdens and adverse drug reactions associated with over-treatment [70]. Furthermore, Marín-Aguilera et al. demonstrated in the lab that HER2DX shows high reproducibility and stabili-

**Table 1.** Gene testing tools used for HR+ breast cancer

| Gene Testing Tool | Number of Genes | Clinical Application | Involved Clinical Trials | References |
|---|---|---|---|---|
| Oncotype DX | 21 | Predicts 10-year recurrence risk in ER+ and LN- patients | In the NSABP B-14 trial, patients receiving endocrine therapy showed 10-year distant recurrence rates of 6.8%, 14.3%, and 30.5% in the low, intermediate, and high-risk groups, respectively ($P < 0.001$). | [61] |
| Prosigna | 55 | Predicts prognosis in postmenopausal women with Stage I or II ER+ and LN+/- breast cancer | In the ABCSG-8 trial, the ROR score significantly enhanced prognostic information beyond clinical predictors ($P < 0.0001$). Luminal A subtype showed a significantly lower 10-year ROR compared to Luminal B ($P < 0.0001$). | [62] |
| MammaPrint | 70 | Predicts distant recurrence risk in Stage I or II ER+/- and LN- patients | MammaPrint demonstrated the strongest prognostic ability for distant metastasis-free survival (dMFS) within the first 5 years after cancer diagnosis (HR=9.6; 95% CI: 4.2-22.1). | [63] |
| EndoPredict | 11 | Predicts 10-year recurrence risk in women with ER+ and LN+/- disease receiving endocrine therapy only | In the ABCSG-6 cohort, the 10-year distant recurrence rates were 8% (3%-13%) in the EP low-risk group and 22% (15%-29%) in the high-risk group ($P < 0.001$); in ABCSG-8, these rates were 6% (2%-9%) and 15% (11%-20%) in the low- and high-risk groups, respectively ($P < 0.001$). | [64] |

**Table 2.** Differences between HER2DX (2022) and HER2DX (2020) content

| | HER2DX (2020) | | HER2DX (2022) | |
|---|---|---|---|---|
| Clinical feature | Tumour size, Nodal status, Number of tumour-infiltrating lymphocytes (TILS), PAM50 subtype | | Tumour size, Nodal status | |
| Genomic feature | Genes associated with better survival outcome (6 genes): | Genes related to luminal differentiation(BAG1) | Immunoglobulin (IGG) module (14 genes): | CD27, CD79A, HLA-C, IGJ, IGKC, IGL, IGLV3-25, IL2RG, CXCL8, LAX1, NTN3, PIM2, POU2AF1 and TNFRSF17 |
| | | Genes related to the normal cell phenotype (KRT5, KRT14, MLPH, MYC) | The tumour cell proliferation signature (4 genes): | EXO1, ASPM, NEK2 and KIF23 |
| | | Basal-like-related genes (PHGDH) | The luminal differentiation signature (5 genes): | BCL2, DNAJC12, AGR3, AFF3 and ESR1 |
| | Genes associated with poor survival outcomes (7 genes): | Genes related to proliferation (CDC6, EXO1, RRM2) | The HER2 amplicon signature (4 genes): | ERBB2, GRB7, STARD3 and TCAP |
| | | Genes related to HER2 amplicion (TMEM45B, FGFR4) | | |
| | | Basal-like-related biology (CDH3) | | |
| | | Genes related to cell invasion (MMP11) | | |
| Application | Predicting survival outcomes | | Predicting survival outcomes, the likelihood of pathological remission from treatment, and assessing ERBB2 expression | |

ty in quantifying the risk of early HER2-positive breast cancer recurrence, the likelihood of pCR, and ERBB2 mRNA expression using Formalin-Fixed, Paraffin-Embedded (FFPE) tumor tissues and purified RNA analysis [71]. The introduction of HER2DX marks a significant advancement towards precision medicine in treating HER2+ breast cancer.

## The development and refinement of HER2DX

### *The emergence of HER2DX (2020)*

In 2020, Prat et al. developed HER2DX (2020), a tool designed to assess whether escalating or de-escalating systemic treatment regimens for early-stage HER2+ breast cancer patients could improve outcomes [59]. These strategies included reducing chemotherapy dosage, shortening the duration of targeted therapy, replacing traditional single-target treatments with dual-target therapies, and substituting monoclonal antibodies with ADC drugs in patients who did not achieve a pCR after neoadjuvant therapy with monoclonal antibodies. The study revealed that most early-stage HER2+ breast cancer patients could achieve desired outcomes with chemotherapy combined with trastuzumab alone, avoiding overtreatment. Further analysis highlighted that traditional parameters, such as tumor size, lymph node status, HR status, and stromal TILs, along with biomarkers like PAM50 subtypes and PIK3CA mutations, were associated with prognosis. Using data from the ShortHER3 clinical trial [72], Pret integrated these factors to form the HER2DX (2020) prognostic model. This model includes tumor size, lymph node status, TILs, subtypes, and 13 genomic markers, and was found to correlate with distant metastasis-free survival (DMFS) in HER2+ patients ($P < 0.0001$). HER2DX (2020) stratified patients into low, medium, and high-risk groups, with distinct 5-year DMFS rates: 98.1%, 88.9%, and 73.9%, respectively, showing significant differences between low and high-risk groups (HR = 0.04, 95% CI: 0.0-0.1, $P < 0.0001$) [59].

### *The refinement of HER2DX (2022)*

As HER2DX (2020) was applied and researched further, Pret et al. identified several limitations: (1) The subjective nature of TILs evaluation, causing inconsistencies across pathologists; (2) HER2DX (2020) assessed only 55 genes, insufficient for a comprehensive genetic tool; (3) With neoadjuvant therapy becoming more common, HER2DX (2020) could not evaluate the pCR rate post-neoadjuvant therapy, limiting its use in guiding comprehensive treatment for early-stage HER2+ patients. Therefore, based on research from various study cohorts and databases, Pret refined HER2DX, resulting in HER2DX (2022) [60].

HER2DX (2022) introduced several improvements compared to its predecessor (**Table 2**). (1) Genetic Markers Expansion: The number of genes analyzed expanded from 55 to 185, enhancing the comprehensiveness of genomic analysis. (2) Adjustment in Scoring for TILs: To address the subjectivity of TILs evaluation [73], HER2DX (2022) adjusted its approach, possibly incorporating quantitative gene expression related to immune infiltration [54, 70, 74]. (3) Optimization of Cut-off Values: HER2DX (2022) optimized cut-off values for risk and pCR scores, offering refined predictions tailored to different patient groups. (4) Increased Focus on Treatment Response Prediction: HER2DX (2022) introduced or refined scores to better predict responses to neoadjuvant and adjuvant therapies. (5) Enhanced Predictive Capabilities for ERBB2 Expression: HER2DX (2022) improved its prediction of ERBB2 expression, aiding in selecting the most suitable patients for anti-HER2 therapies. (6) Application to a Broader Range of Clinical Scenarios: HER2DX (2022) extended its applicability to include patients with advanced disease. (7) Validation Across Diverse Populations: HER2DX (2022) was validated across a more diverse population, enhancing its global utility.

HER2DX (2022) is developed based on two clinical-pathological features and 27 genes, divided into four genetic marker groups, aiming to predict survival outcomes, distant relapse risk, and treatment response in HER2+ breast cancer patients using three scoring systems: the HER2DX Risk Score, the HER2DX pCR Score, and the HER2DX ERBB2 Expression Score, thus aiding treatment decisions (**Figure 2**). The two clinical features include tumor size and lymph node staging. The four genetic groups in HER2DX include 14 immune infiltration-related genes (CD27, CD79A, HLA-C, IGJ, IGKC, IGL, IGLV3-25, IL2RG, CXCL8, LAX1, NTN3, PIM2,

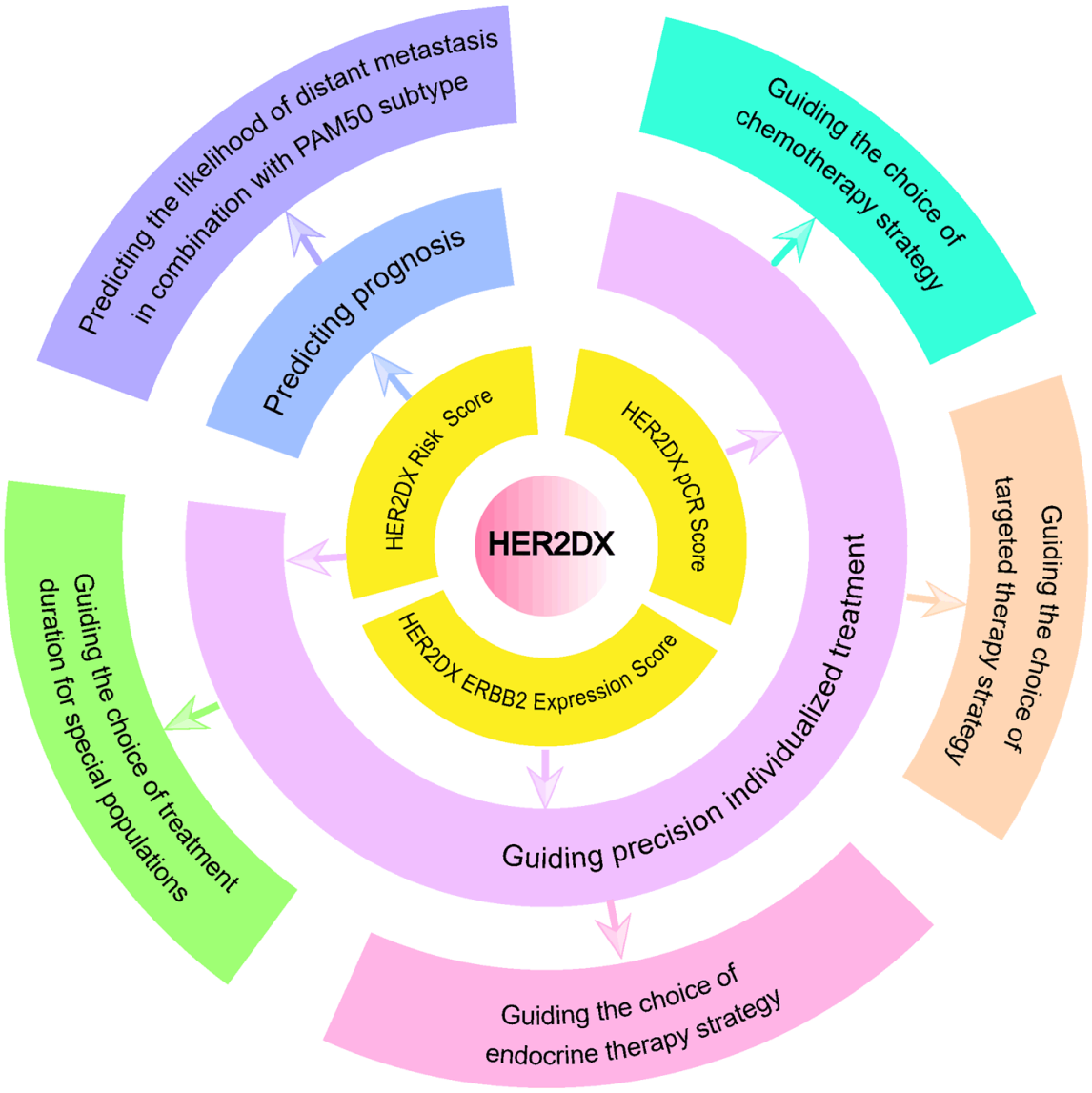


**Figure 2.** The application of HER2DX in clinical practice.

POU2AF1, and TNFRSF17), 4 tumor cell proliferation-related genes (EXO1, ASPM, NEK2, and KIF23), 5 luminal differentiation-related genes (BCL2, DNAJC12, AGR3, AFF3, and ESR1), and 4 HER2 amplicon-related genes (ERBB2, GRB7, STARD3, and TCAP). Pret et al. [60] developed the three scoring systems by integrating data from retrospective studies, prospective studies, and international database data. HER2DX pCR Likelihood Score: developed within the H. Clinic HER2+ cohort, this score is based on HER2, IGG, luminal and proliferation signatures, tumor stage, and nodal stage. It was validated and explored in multiple cohorts (PAMELA/H.Clinic/Padova HER2+, CALGB-40601, and ISPY-2 cohorts), showing a significant correlation with pCR as both a continuous and categorical variable. Group cutoff values were established as low-pCR groups (0, 33.3), medium-pCR groups (33.3-66.7), and high-pCR groups (66.7-100). HER2DX Risk Score: Developed in the Short-HER HER2+ cohort, this score is primarily based on IGG, luminal and proliferation signatures, tumor stage, and nodal stage. It was validated across various cohorts (H.Clinic/Padova/PAMELA HER2+ cohorts and TCGA, METABRIC, SCAN-B, CALGB-40601 databases), showing a significant correlation with

**Table 3.** Summary of studies related to HER2DX

| Clinicaltrials Number | Study | Molecular typing | Number of analyzed samples | References |
|---|---|---|---|---|
| NCT02411344 | PerELISA | HR+ HER2+ | 55 | [75] |
| NCT01853748 | ATEMPT | HER2+ | 187 | 2022 SABCS Abstract PD18-01 |
| NCT00542451 | APT | HER2+ | 284 | [76] |
| NCT03716180 | DAPHNe | HER2+ | 80 | [77] |
| NCT00770809 | CALGB-40601 | HER2+ | 263 | [78, 79] |
| NCT01973660 | PAMELA | HER2+ | 91 | [79, 80] |
| NCT01042379 | ISPY-2 | HER2+ | 127 | [79, 81] |
| NCT05912062 | BiOnHER | HER2+ | 46 | [79] |
| | NEOHER | HER2+ | 67 | [79] |
| | GOM | HER2+ | 155 | [79, 82] |

disease-free survival (DFS), overall survival (OS), and other prognostic factors. Group cutoff values were established as low-risk groups (0, 50) and high-risk groups (50, 100). HER2DX ERBB2 Expression Score: Developed in the Short-HER and H.Clinic cohorts and validated in multiple HER2+ cohorts (H.Clinic/Padova/PAMELA HER2+ and SOLTI HER2- cohorts), this score is correlated with the clinical HER2 status in HER2+ breast cancer patients. An optimal cutoff value of -0.98 was determined using Youden's analysis to predict HER2 receptor status.

These scoring systems reveal the long-term risk of recurrence and metastasis, the probability of pCR, and the tumor HER2 expression level, enabling more accurate treatment decisions and survival predictions. HER2DX is currently being applied and evaluated in multiple clinical studies (**Table 3**).

### The prognostic value of HER2DX

The HER2DX Risk Score, whether analyzed as a continuous or categorical variable, is statistically associated with survival outcomes and distant relapse in HER2+ breast cancer patients. This score predicts survival and the likelihood of relapse and distant metastasis across different patient populations following systemic therapy [59, 60]. Villacampa et al. evaluated survival in patients treated with trastuzumab, followed up long-term within the combined NEOHER and PAMELA cohorts [79]. They categorized patients using HER2DX Risk Score cutoff values. Among those achieving pCR, the 6-year event-free survival (EFS) was 98.1% in the low-risk group compared to 89.4% in the high-risk group. For patients not achieving pCR, the 6-year EFS was 93.5% in the low-risk group versus 78.8% in the high-risk group. Overall, the HER2DX Risk Score, as a categorical variable, correlated with EFS ($P < 0.001$) and OS ($P = 0.006$), helping to identify patients at low recurrence risk. Similarly, in the SCAN-B dataset [83], Villacampa et al. found a statistically significant correlation between HER2DX Risk Score, as a continuous variable, and OS (HR = 1.31 per 10-unit increment, 95% CI: 1.13-1.51, $P < 0.001$). As a categorical variable, 7-year OS was 94.5% in the low-risk group and 78.6% in the high-risk group (HR = 3.87, 95% CI: 2.26-6.65, $P < 0.001$). In the APT phase II clinical trial [76], Tolaney et al. used HER2DX to assess the association between HER2DX Risk Score and invasive disease-free survival (iDFS) and relapse-free interval (RFI). They found that HER2DX Risk Score, as a continuous variable, significantly correlated with iDFS ($P = 0.047$) and RFI ($P = 0.011$). Grouping patients by a predefined HER2DX Risk Score cutoff of 50 revealed a significant increase in recurrence risk in the high-risk group. However, using the Contal and O'Quigley method [84], the optimal cutoff to differentiate low and high-risk patients in the APT trial was determined to be a HER2DX Risk Score of 32. Patients with a score below 32 had a 1.4% probability of recurrence after 10 years, compared to 13.3% for those with a score of 32 or higher. The HER2DX genomic tool accurately identifies patients at increased recurrence risk. For these higher-risk patients, it is advisable to shorten follow-up intervals, monitor them closely, and conduct regular exams to prevent and detect recurrence early for timely intervention.

Although advances in anti-HER2 therapy have significantly improved the prognosis of patients with HER2+ breast cancer, more than 10% of these patients may still develop distant metastases during follow-up [49, 85]. Further research has shown that the HER2DX Risk Score, in combination with the PAM50 subtype, can predict the likelihood of distant metastasis. Dieci et al. demonstrated that the HER2DX Risk Score is associated with the risk of any distant metastasis: the 10-year cumulative incidence of relapse and metastasis was 19.7% for patients with high risk scores and 5.3% for those with low risk scores ($P < 0.001$) [86]. The combination of the HER2DX Risk Score and the PAM50 subtype can predict the likelihood of specific metastatic sites. Tumors of the HER2-enriched intrinsic molecular subtype are more likely to metastasize to the brain, basal-like tumors are associated with an increased risk of lung metastasis, and luminal tumors are more prone to bone metastasis. When combining the HER2DX Risk Score with PAM50 subtypes, Luminal A subtype showed a lower incidence of any distant metastasis in both low and high HER2DX risk groups, although this difference was not statistically significant. In terms of brain metastasis, the cumulative incidence was very low in the low HER2DX risk group, regardless of the intrinsic subtype. In contrast, in the high HER2DX risk score group, patients with HER2-enriched subtype had a significantly higher incidence of brain metastasis compared to other subtypes. For lung metastasis, the incidence was significantly higher in basal-like subtypes than in other subtypes, both in low and high HER2DX risk groups [86]. These results indicate that patients with a low HER2DX Risk Score and Luminal A tumors have a very low probability of developing metastasis within 10 years, suggesting that these patients may not require intensified follow-up. For other patient groups classified by combining HER2DX and PAM50 subtypes, intensified follow-up targeted at specific potential metastatic sites and corresponding intensified treatment measures may be necessary.

## HER2DX-guided precision individualized comprehensive treatment

### *HER2DX pCR score predicts the likelihood of pCR in anti-HER2 neoadjuvant therapy*

The HER2DX pCR Score, both as a continuous and categorical variable, is statistically correlated with the pCR rate in HER2+ breast cancer patients [60]. Research by Villacampa et al. demonstrated that across different patient subgroups from clinical trials such as ISPY-2, CALGB-40601, DAPHNe, GOM, BiOnHER, NEOHER, and PAMELA, the HER2DX pCR Score was significantly associated with pCR. Specifically, the pCR rates for low, medium, and high HER2DX pCR groups were 20.2%, 55.3%, and 74%, respectively, indicating that patients with higher HER2DX pCR Scores are more likely to achieve pathological complete remission, regardless of the treatment modality used [79]. Therefore, grouping HER2+ breast cancer patients based on the HER2DX pCR Score can guide the selection of different treatment plans, enhancing the personalization and effectiveness of therapy.

### *Guiding the personalized selection of ADCs*

Research by Brasó-Maristany et al. indicated that in second-line treatment of advanced HER2+ breast cancer, the HER2DX ERBB2 expression score correlates with response to T-DM1 therapy. The overall response rates to T-DM1 in the low, medium, and high HER2DX ERBB2 expression groups were 0%, 29%, and 56%, respectively ($P < 0.001$) [87]. Similarly, Villacampa et al. found in the SCAN-B dataset that patients with low HER2DX ERBB2 scores showed no significant benefit from T-DM1 (HR = 1.00, 95% CI: 0.21-4.77). However, significant benefits were observed in patients with medium (HR = 0.10, 95% CI: 0.01-0.92) and high (HR = 0.15, 95% CI: 0.10-0.23) HER2DX ERBB2 scores [83]. Patients in the medium/high HER2DX ERBB2 score groups may be ideal candidates for T-DM1, which, compared to T-DXd, offers higher efficacy, lower cost, and reduced toxicity. Considering efficacy, economic costs, and safety, T-DM1 is a strong treatment option for patients with high HER2DX ERBB2 scores.

### *Personalized choice of anti-HER2 therapy combined with chemotherapy*

Chemotherapy drugs, such as anthracyclines, have significant side effects, potentially causing cardiotoxicity, neutropenia, diarrhea, and other adverse events [88-90]. Although the combination of trastuzumab monoclonal antibody with chemotherapy remains the first-line choice for many patients with HER2+ breast cancer [23], for some patients, the use of vari-

ous combination treatments or chemotherapy drugs may constitute overtreatment. The HER2DX pCR Score plays an essential role in this regard, as it can effectively select those patient groups who can still achieve the expected treatment outcomes with reduced or even no chemotherapy drug use. This precise treatment selection helps to reduce adverse events and improve the quality of life for patients. Through the HER2DX pCR Score, physicians can more accurately assess patients' responses to chemotherapy, thereby developing more suitable and personalized treatment plans for patients with HER2+ breast cancer. This approach not only improves treatment efficacy but also reduces the physical burden on patients, enhancing their overall treatment experience.

Villacampa et al. reported that, within cohorts receiving trastuzumab monoclonal antibody combined with multi-drug chemotherapy, single-agent taxane, or no chemotherapy, grouping by the HER2DX pCR Score showed differences in pCR rates between multi-drug chemotherapy and single-agent taxane as -4.5%, 25.5%, and -3.2% across different pCR score groups. The increase in pCR rate due to multi-drug chemotherapy was statistically significant only in tumors of the HER2DX medium pCR group (OR = 3.11, 95% CI: 1.54-6.49, P = 0.002) [79]. This suggests that for patients in the HER2DX medium pCR group, multi-drug chemotherapy could lead to a higher pCR rate, while for patients in the high and low pCR groups, the effect of multi-drug chemotherapy was not significantly superior to single-agent taxane. This finding is significant for the treatment strategy of patients with HER2+ breast cancer. The HER2DX pCR Score can help identify patients who may benefit more from a dual anti-HER2 combination with single-agent taxane, especially those with a medium HER2DX pCR score. For patients with high or low HER2DX pCR scores, using multi-drug chemotherapy does not significantly improve the pCR rate and may increase unnecessary toxic side effects. Furthermore, the PerELISA trial results showed that without using chemotherapy, the HER2DX ERBB2 Expression Score was significantly associated with pCR (P = 0.003), and this relationship was independent of the clinical HER2 immunohistochemistry level (2+ vs 3+) [75]. This suggests that patients with a high HER2DX ERBB2 Expression Score have a higher likelihood of achieving pCR after anti-HER2 therapy and can opt for dual-targeted therapy to avoid chemotherapy.

*Personalized choice between dual anti-HER2 therapy and single Anti-HER2 therapy*

Previous studies have shown that single anti-HER2 therapy with trastuzumab monoclonal antibody combined with chemotherapy achieved a pCR rate of 29-46% [78, 91, 92]. The addition of a second anti-HER2 drug, such as pertuzumab monoclonal antibody or lapatinib, increased the pCR rate by 10-20% [85, 93], with a slight improvement in long-term survival rates. However, this raises a critical question: can all HER2+ breast cancer patients benefit from dual anti-HER2 therapy? This is where the HER2DX pCR Score demonstrates its value. This score effectively identifies patient groups most likely to benefit from dual anti-HER2 therapy.

Villacampa et al. categorized patients into high, medium, and low pCR groups based on the HER2DX pCR Score. The differences in pCR rates between patients receiving trastuzumab monoclonal antibody combined with chemotherapy and those receiving dual anti-HER2 therapy combined with chemotherapy were 17.6%, 5.4%, and 4.6% across these groups, respectively, with a statistically significant difference only in the high HER2DX pCR group (OR = 2.36, 95% CI: 1.09-5.42, P = 0.03) [79]. The HER2DX pCR Score can identify those in the high HER2DX pCR group who would benefit from dual anti-HER2 therapy. The study's results suggest that the HER2DX pCR Score can help physicians determine which HER2+ breast cancer patients might benefit from dual anti-HER2 therapy. For patients in the high pCR group, dual therapy might be more suitable, while for those in the medium and low pCR groups, considering the economic cost and potential toxic side effects of dual therapy, single anti-HER2 therapy might be a more reasonable choice.

*Personalized endocrine therapy choices for HER2+/HR+ patient*

The HER2+/HR+ subtype of breast cancer constitutes about 70% of HER2+ cases [94]. This

subtype is generally more aggressive and associated with a poorer prognosis compared to HR-negative (HR-) breast cancer [95]. The complexity of treating HER2+/HR+ breast cancer lies in the potential insensitivity to anti-HER2 therapy, possibly due to interactions between the estrogen receptor (ER) pathway and the HER2 pathway [96]. In treating HER2+/HR+ breast cancer, it is often necessary to consider both anti-HER2 therapy and endocrine therapy. However, resistance to endocrine therapy can be a significant challenge. The HER2DX pCR Score is crucial here, as it can help predict which HER2+/HR+ patients may respond well to endocrine therapy, guiding treatment decisions effectively.

In the perELISA phase III clinical trial [75], pretreatment biopsies were conducted on HER2+/HR+ patients to assess the Ki-67 index, followed by another biopsy after 2 weeks of letrozole treatment. This was to evaluate changes in Ki-67 and distinguish between estrogen-sensitive disease (ESD) and estrogen-resistant disease (ERD). The HER2DX pCR Score showed a significant correlation with the Ki-67 response after letrozole treatment ($P = 0.002$). The response rates (a reduction of more than 20% in baseline Ki-67 levels) were 89.7%, 65.0%, and 16.7% in the low, medium, and high HER2DX pCR score groups, respectively. This suggests that a lower HER2DX pCR Score predicts a better response to letrozole, while a higher score indicates reduced tumor sensitivity to endocrine therapy. Additionally, the study revealed that the pCR rate in ESD patients was 22.5%, significantly lower than the 80% observed in ERD patients. The HER2DX pCR Score was significantly correlated with the pCR rate in ESD patients ($P = 0.008$), with rates of 7.7%, 46.2%, and 100.0% in the low, medium, and high HER2DX pCR score groups, respectively ($P < 0.004$). However, the score was not significantly associated with pCR in ERD patients ($P = 1$). These findings suggest that the HER2DX pCR Score can not only predict early response to letrozole monotherapy but also help identify patients who may benefit from combined endocrine and anti-HER2 therapy. For ESD patients with a lower HER2DX pCR Score, escalated treatment may be necessary to improve outcomes.

*HER2DX for predicting prognosis in special populations and personalizing de-escalation treatment choices*

Research on trastuzumab therapy duration has shown that long-term treatment (12 months) offers a lower risk of recurrence compared to short-term treatment (e.g., 9 weeks or 6 months), but with an increased risk of side effects like cardiotoxicity [72, 97-99]. Exploratory research within the Short-Her trial suggested that a low HER2DX Risk Score might guide the choice of treatment regimen for specific patient groups [60, 72]. Patients with a low HER2DX Risk Score, particularly those with significant comorbidities or a history of cardiotoxicity, might be suitable candidates for short-term trastuzumab therapy. This finding helps physicians create more personalized treatment plans for HER2+ breast cancer patients, aiming for optimal outcomes while minimizing side effects.

## Conclusion and future perspective

The HER2DX pCR Score and Risk Score, despite their weak correlation (correlation coefficient about -0.19) [60], provide complementary information critical for guiding treatment decisions and selecting appropriate escalation or de-escalation strategies. The core value of HER2DX lies in its pCR and Risk Scores, while the HER2DX ERBB2 Expression Score serves mainly as supplementary information. Although most early-stage HER2+ breast cancer patients can achieve treatment goals with trastuzumab and chemotherapy, the effectiveness of different drug combinations varies. HER2DX can help predict pCR rates and survival outcomes after various treatments, offering personalized treatment options: for some patients, de-escalation (e.g., reducing chemotherapy types or cycles) may achieve similar therapeutic effects as standard treatment, minimizing toxicity and adverse events, thus avoiding overtreatment. Other patients may require escalated treatment (e.g., adding more effective drugs or extending treatment duration) to achieve expected results, especially when trastuzumab and chemotherapy alone do not meet therapeutic needs. HER2DX provides a tool for making more precise treatment choices based on individual patient conditions, predicting survival outcomes, recurrence, and the risk of distant

metastasis, guiding follow-up intervals, and enabling more effective treatment and monitoring.

As the field of HER2+ breast cancer treatment advances, challenges remain for specific patients. Long-term use of the same anti-HER2 therapy may lead to disease progression or ineffective outcomes when switching therapies. This could be due to tumor biology, individual patient differences, or resistance development. Resistance is a crucial issue in HER2+ breast cancer treatment, potentially caused by genetic mutations, expression changes, or signaling pathway alterations, rendering previously effective treatments ineffective. The HER2DX model, while effective in predicting pCR, may also help predict drug resistance. Although research in this area is limited, the correlation between drug resistance and reduced pCR rates suggests HER2DX's potential in predicting resistance. Further research into the genes within the HER2DX model and their roles in resistance mechanisms could unveil new therapeutic targets and strategies to overcome resistance.

Despite the promising results, HER2DX has limitations that future research must address. (1) Grouping Threshold Values: Different trials may identify different optimal cutoff values. For example, the APT trial found "32" as the optimal HER2DX Risk Score cutoff, differing from the standard threshold, suggesting the need for score adjustments based on patient populations and treatment contexts. (2) Geographical Applicability and Case Numbers: HER2DX has been evaluated in over 2,000 patients, with relatively few Chinese participants. Genetic and environmental differences suggest that effectiveness may vary between Asian and Western populations, necessitating broader evaluations. (3) Applicability to Advanced Breast Cancer: HER2DX was developed for early-stage HER2+ breast cancer. Its accuracy and applicability in advanced cases, particularly for predicting systemic treatment outcomes, may require further validation. (4) Validation Scope for Dual Anti-HER2 Therapy: HER2DX primarily validated trastuzumab and pertuzumab combination therapy, possibly overlooking the roles of TKIs and ADCs in dual-targeted therapy. Evaluating HER2DX in broader treatment regimens is necessary. (5) Lack of Prospective Studies: Most HER2DX research is retrospective, with few prospective studies that are crucial for validating its clinical applicability and effectiveness. (6) Cost Issues: HER2DX, based on genetic testing, may involve higher costs, limiting its widespread clinical use. Cost-effectiveness and financial burden must be considered.

Future research should address these limitations to enhance HER2DX's clinical value. With more studies, HER2DX can be optimized for broader drug combinations, patient groups, and treatment stages. As new drugs and strategies emerge, HER2DX may evolve to meet new clinical challenges.

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## Disclosure of conflict of interest

None.

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