Assessment of the HER2DX Assay in Patients With ERBB2-Positive Breast Cancer Treated With Neoadjuvant Paclitaxel, Trastuzumab, and Pertuzumab

Adrienne G. Waks, MD; Esther R. Ogayo, BS; Laia Paré, PhD; Mercedes Marin-Aguilera, PhD; Fara Brasó-Maristany, PhD; Patricia Galván, PhD; Oleguer Castillo, MS; Olga Martínez-Sáez, MD; Ana Vivancos, PhD; Patricia Villagraña, PhD; Guillermo Villacampa, MSc; Paolo Tarantino, MD; Neelam Desai, MD; Jennifer Guerriero, PhD; Otto Metzger, MD; Nadine M. Tung, MD; Ian E. Krop, MD; Joel S. Parker, PhD; Charles M. Perou, PhD; Eric P. Winer, MD; Sara M. Tolaney, MD, MPH; Elizabeth A. Mittendorf, MD, PhD

IMPORTANCE Patients with early-stage ERBB2 (formerly HER2)-positive breast cancer (ERBB2+ BC) who experience a pathologic complete response (pCR) after receiving neoadjuvant therapy have favorable survival outcomes. Predicting the likelihood of pCR may help optimize neoadjuvant therapy.

OBJECTIVE To test the ability of the HER2DX assay to predict the likelihood of pCR in patients with early-stage ERBB2+ BC who are receiving deescalated neoadjuvant therapy.

DESIGN, SETTING, AND PARTICIPANTS In this diagnostic/prognostic study, the HER2DX assay was administered on pretreatment tumor biopsy samples from patients enrolled in the single-arm, multicenter, prospective phase 2 DAPHNe clinical trial who had newly diagnosed stage I to III ERBB2+ BC that was treated with neoadjuvant paclitaxel weekly for 12 weeks plus trastuzumab and pertuzumab every 3 weeks for 4 cycles.

INTERVENTIONS AND EXPOSURES The HER2DX assay is a classifier derived from gene expression and limited clinical features that provides 2 independent scores to predict prognosis and likelihood of pCR in patients with early-stage ERBB2+ BC. The assay was administered on baseline tumor samples from 80 of 97 patients (82.5%) in the DAPHNe trial.

MAIN OUTCOMES AND MEASURES The primary aim was to test the ability of the HER2DX pCR likelihood score (as a continuous variable from 0-100) to predict pCR (ypT0/isN0).

RESULTS Of 80 participants, 79 (98.8%) were women and there were 4 African American (5.0%), 6 Asian (7.5%), 4 Hispanic (5.0%), and 66 White individuals (82.5%); the mean (range) age was 50.3 (26.0-78.0) years. The HER2DX pCR score was significantly associated with pCR (odds ratio, 1.05; 95% CI, 1.03-1.08; P < .001). The pCR rates in the HER2DX high, medium, and low pCR score groups were 92.6%, 63.6%, and 29.0%, respectively (high vs low odds ratio, 30.6; P < .001). The HER2DX pCR score was significantly associated with pCR independently of hormone receptor status, ERBB2 immunohistochemistry score, HER2DX ERBB2 expression score, and prediction analysis of microarray 50 ERBB2-enriched subtype. The correlation between the HER2DX pCR score and prognostic risk score was weak (Pearson coefficient, −0.12). Performance of the risk score could not be assessed due to lack of recurrence events.

CONCLUSIONS AND RELEVANCE The results of this diagnostic/prognostic study suggest that the HER2DX pCR score assay could predict pCR following treatment with deescalated neoadjuvant paclitaxel and pertuzumab in patients with early-stage ERBB2+ BC. The HER2DX pCR score might guide therapeutic decisions by identifying patients who are candidates for deescalated or escalated approaches.
Patients with stage II and III ERBB2-positive (ERBB2+; formerly HER2) breast cancer typically receive neoadjuvant chemotherapy with ERBB2-directed therapy. Those experiencing a pathologic complete response (pCR) have significantly better survival outcomes than those with residual invasive disease1,2; therefore, they may be candidates for de-escalation of therapy. The HER2DX assay is a supervised learning algorithm that incorporates clinical information (tumor size, nodal staging) and 4 gene expression signatures (immune infiltration, tumor cell proliferation, luminal differentiation, and ERBB2 amplicon expression) to provide 2 independent scores with the potential to predict the likelihood of pCR (pCR score) and long-term prognosis (risk score).3,4

DAPHNe (NCT03716180) was a single-arm phase 2 trial in which patients with stage II to III ERBB2+ breast cancer received a deescalated neoadjuvant regimen comprising paclitaxel, trastuzumab, and pertuzumab (THP). The overall pCR rate was 56.7%.5 Patients received adjuvant ERBB2-directed therapy with or without further chemotherapy based on their response to the neoadjuvant regimen; adjuvant trastuzumab and pertuzumab only was recommended for patients who experienced pCR. In the present study, the HER2DX assay was administered on pretreatment tumor biopsy specimens to investigate the predictive value of the HER2DX pCR score, evaluate the HER2DX pCR score assay according to hormone receptor (HR) status, and explore the association between the predictive HER2DX pCR score and the prognostic HER2DX risk score. The prognostic value of the risk score could not be assessed given lack of recurrence events in the trial.

Methods

DAPHNe Patient Population and Trial Therapy
DAPHNe was a single-arm prospective phase 2 trial that enrolled patients with stage II to III ERBB2+ breast cancer. All patients received neoadjuvant THP for 4 cycles before surgery. Five patients (5.1%) experienced incomplete clinical response to THP and received additional neoadjuvant chemotherapy; they were excluded from this analysis. The degree of response to neoadjuvant therapy was quantified by the residual cancer burden (RCB) score;5 pCR was defined as an RCB score of 0 (ypT0/isN0). All trial procedures were approved by the Dana-Farber/Harvard Cancer Center institutional review board, all patients provided written informed consent, and the study conformed to the Standards for Reporting of Diagnostic Accuracy (STARD) 2015 reporting guideline.7

HER2DX Assay
Development of the HER2DX assay was described previously.3 The HER2DX assay incorporates limited clinical features and expression of 27 genes that encompass immune infiltration, tumor cell proliferation, luminal differentiation, and ERBB2 amplicon expression signatures. The assay provides 2 independent scores to predict prognosis (risk score) and the likelihood of pCR (pCR score). The HER2DX assay also produces an ERBB2 score based on the level of ERBB2 gene expression.

Scores are continuous, as well as subdivided into ordinal groups based on previously reported cut points.3

Application of HER2DX to DAPHNe Tumor Samples and Statistical Methods
Ribonuclease acid was extracted from baseline tumor biopsy specimens. The HER2DX assay was retrospectively evaluated centrally. Univariate and multivariable logistic regression analyses were used to investigate the association of each variable with pCR. The least absolute shrinkage and selection operator regression was used for variable selection in the multivariate model. Receiver operating characteristic (ROC) curves were used as a performance measure. Statistical analyses were performed in R, version 4.0.5 (R Foundation), and data imputation for the multivariable analysis was performed using multivariate imputation (mice R package). No adjustments were made for multiple comparisons, and the significance level was set to 2-sided α = .05.

Results

Patient and Tumor Characteristics
The HER2DX assay was administered for 80 of 98 patients (81.6%) enrolled and treated during the trial (eFigure in Supplement 1). Clinical T2 to T3 disease represented 65 cases (81.3%); 52 patients (65.0%) had clinical node-negative disease, and 56 (70.0%) had HR-positive disease. eTable 1 in Supplement 1 compares the HER2DX assay population with the overall trial population. The pCR rate among the HER2DX cohort was 60.0% (95% CI, 49.3%-70.7%). The pCR rate was 87.0% (95% CI, 79.3%-94.4%) in patients with HR-negative disease and 48.2% (95% CI, 37.2%-59.1%) in patients with HR-positive disease. The proportion of patients in the HER2DX pCR score high, medium, and low categories was 33.7%, 27.5%, and 38.8%, respectively.

Performance of the HER2DX pCR Score for pCR Prediction
The HER2DX pCR score as a continuous variable was significantly associated with pCR (odds ratio, 1.05; 95% CI, 1.03-1.08; P < .001), with a receiver operating characteristic curve.

Key Points

Question Can the HER2DX assay predict pathologic complete response (pCR) in patients with early-stage ERBB2 (formerly HER2)-positive breast cancer who were treated with neoadjuvant paclitaxel, trastuzumab, and pertuzumab?

Findings In this diagnostic study of biopsy specimens from 80 patients with early-stage ERBB2-positive breast cancer, the HER2DX assay was administered on baseline tumor biopsy specimens from patients treated during the phase 2 DAPHNe clinical trial. In a multivariable model that incorporated established predictive gene expression–based and clinicopathologic variables, including hormone receptor status, the HER2DX pCR likelihood score was significantly associated with pCR.

Meaning The study results suggest that the HER2DX assay may help to optimize escalation or deescalation of neoadjuvant therapy in patients with early-stage ERBB2-positive breast cancer.
Area under the curve of 0.84 (95% CI, 0.75-0.92) for performance of HER2DX pCR score (Figure, A). The Figure depicts the expression of the 4 gene expression signatures across the HER2DX pCR score high, medium, and low groups. The pCR rates in the HER2DX pCR score high, medium, and low groups were 92.6%, 63.6%, and 29.0%, respectively (odds ratio of 30.6 for comparison of pCR score high vs pCR score low; 95% CI, 6.0-156.9; \( P < .001 \); Table). eTable 2 in Supplement 1 shows RCB categories according to HER2DX pCR score. In a univariable analysis evaluating standard clinicopathologic variables and various expression-based classifiers, there were multiple significant predictors of pCR status, including HER2DX pCR score, HER2DX ERBB2 score, prediction analysis of microarray 50 ERBB2-enriched status, ERBB2 immunohistochemistry status, and HR status. In a multivariable analysis, HER2DX pCR score and ERBB2 score were the only significant predictors of pCR (Table). The HER2DX pCR score performed well in HR-negative and HR-positive subpopulations (receiver operating characteristic curve areas under the curve; IGG immunoglobulin G; IHC, immunohistochemistry).

**Figure. Performance and Genomic Features of the HER2DX Pathologic Complete Response (pCR) Score for Predicting pCR Following Neoadjuvant Paclitaxel, Trastuzumab, and Pertuzumab (THP) Therapy**

<table>
<thead>
<tr>
<th>HER2DX pCR score</th>
<th>HER2DX pCR groups</th>
<th>HR IHC status</th>
<th>pCR status</th>
<th>Nodal status</th>
<th>Tumor size</th>
<th>HER2DX pCR score</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>pCR-high</td>
<td>HR-negative</td>
<td>no pCR</td>
<td>N0</td>
<td>T1</td>
<td>2.0</td>
</tr>
<tr>
<td>1.0</td>
<td>pCR-medium</td>
<td>HR-positive</td>
<td>pCR</td>
<td>N1</td>
<td>T2</td>
<td>1.0</td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td>N2</td>
<td>T3</td>
<td>0.0</td>
</tr>
<tr>
<td>-1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.0</td>
</tr>
<tr>
<td>-2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.0</td>
</tr>
</tbody>
</table>

Receiver operating characteristic curve analysis of the HER2DX pCR score among all patients (A), hormone receptor (HR)–negative patients (B), and HR-positive patients (C). D, Expression of the 4 HER2DX gene expression signatures (immune, proliferation, luminal, and ERBB2 [formerly HER2] amplicon) across the HER2DX pCR score high, medium, and low groups. AUC indicates area under the curve; IGG immunoglobulin G; IHC, immunohistochemistry.

**HER2DX Risk Score Categories**

The proportion of patients grouped into the HER2DX high vs low risk score groups was 48.7% and 51.3%, respectively. The correlation between the HER2DX pCR score and risk score was weak (Pearson coefficient, \(-0.12\)). With 19.1 (IQR, 15.2-22.5) months of median follow-up, there were no breast cancer recurrences, so HER2DX risk score performance could not be assessed.
Discussion

To our knowledge, the results of this study represent the first validation of HER2DX pCR score as a predictive assay in patients with ERBB2+ breast cancer who were treated with neoadjuvant THP. They also highlight the ability of HER2DX pCR score to outperform established predictive biomarkers, such as ERBB2-enriched subtype, and demonstrate the ability of HER2DX to predict pCR in HR-positive and HR-negative patient populations despite the well-documented differences in responsiveness to neoadjuvant chemotherapy plus ERBB2-directed therapy between those subgroups.

Treatment with THP as a deescalated neoadjuvant regimen is the focus of 2 ongoing prospective clinical trials: CompassHER2-pCR (NCT04266249) and DECRESCENDO (NCT04675827). While the pCR-based deescalation approach is currently experimental, if the primary objectives of these 2 trials are met, neoadjuvant THP will likely become a standard regimen for patients with early-stage ERBB2+ breast cancer. The DAPHNe trial cohort, in combination with 2 other cohorts that included patients treated with neoadjuvant taxane/trastuzumab with or without pertuzumab, confirmed that a high HER2DX pCR score could predict a high likelihood of pCR following neoadjuvant THP and benefit from pertuzumab specifically.8 As adjuvant escalation options for ERBB2+ breast cancer may become further intensified, the customization of neoadjuvant therapy to optimize pCR is increasingly important. With further study as a predictive biomarker across neoadjuvant regimens, combined with further validation of the companion prognostic assay, HER2DX pCR score may identify candidates for deescalation beyond THP and escalation from THP in the neoadjuvant setting.

Limitations

This study had several limitations. Biomarker analyses were performed in retrospective fashion, and only a subset of the over-
all trial population was evaluable for the HER2DX biomarker (although this subpopulation appeared similar to the overall trial population). The HER2DX prognostic risk score could not be evaluated due to the lack of recurrence events in this cohort.

Conclusions

To date, genomic risk scores have only played a routine role in managing HR-positive/ERBB2-negative breast cancer.

However, individualization of therapy is increasingly important in the management of early-stage ERBB2+ breast cancer as therapeutic options expand with newer agents being evaluated in ongoing clinical trials. Introduction of a pCR-predictive and long-term prognostic risk score is needed. The findings of this prognostic/diagnostic study indicate that prospective incorporation of HER2DX into escalation and de-escalation trial designs can potentially further define the role for this assay in managing early-stage ERBB2+ breast cancer.


