JAMA Oncology | Brief Report

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

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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 II 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 with trastuzumab 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.

Related article

Supplemental content

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JAMA Oncol. doi:10.1001/jamaoncol.2023.0181
Published online April 27, 2023.

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 disease[1,2]; therefore, they may be candidates for deescalation 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.[5] 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.

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.

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.[5] 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[6]; 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

Ribonucleic 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 $\alpha = .05$.

Results

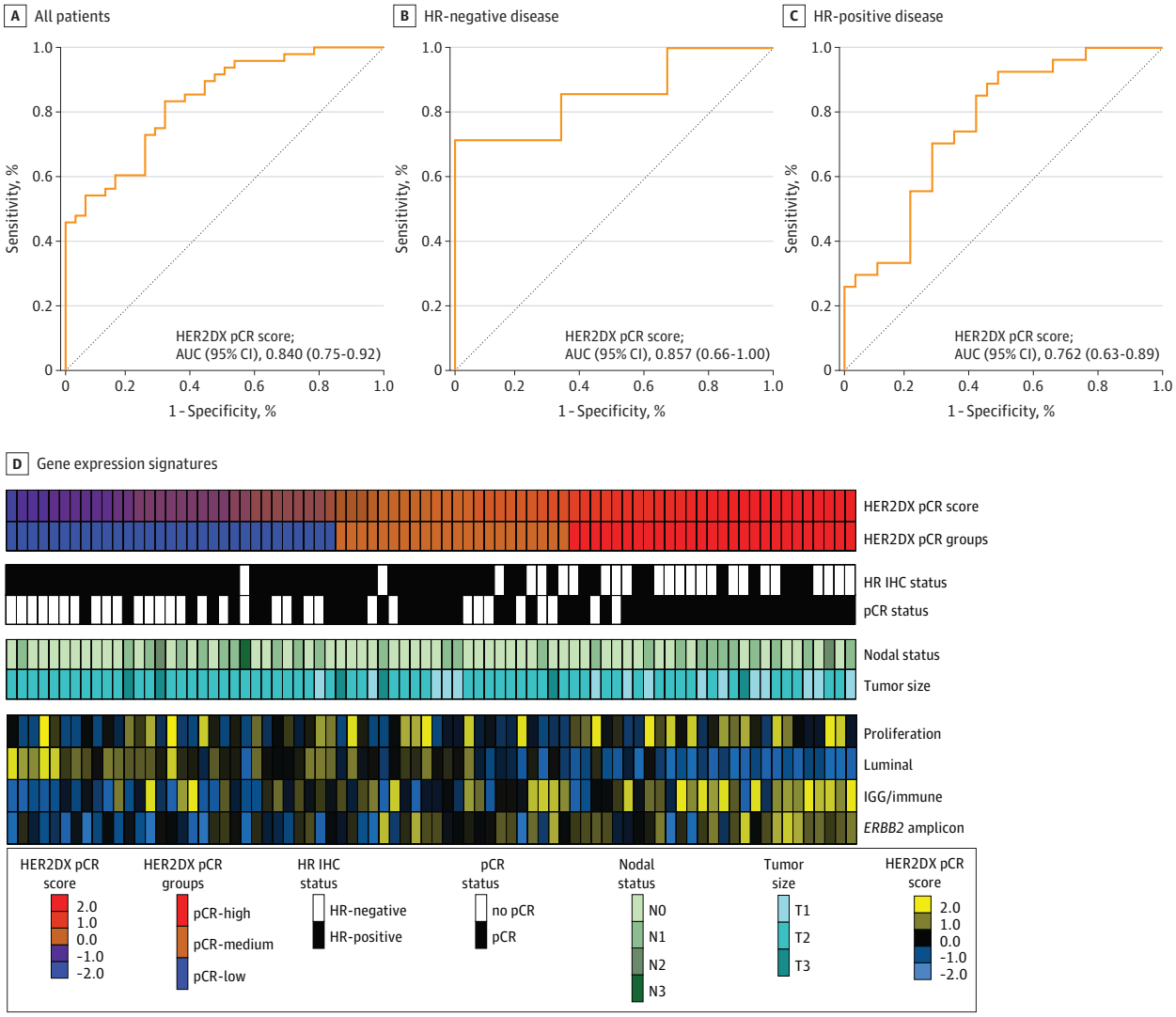
Patient and Tumor Characteristics

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, 79.6%-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

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



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.

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 of 0.857 and 0.762, respectively) (Figure, B and C). The pCR rates by HR status and HER2DX pCR score group are shown in eTable 3 in Supplement 1.

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,[5] so HER2DX risk score performance could not be assessed.

Table. Association of Pretreatment Baseline Variables With pCR in 80 Patients With *ERBB2*-Positive Early-Stage Breast Cancer Treated With Neoadjuvant THP in the DAPHNe Clinical Trial

Characteristic	No.	pCR rate, %	Univariable		Multivariable	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value
Overall cohort	80	60.0				
HER2DX pCR score (continuous variable)	80	NA	1.05 (1.03-1.08)	<.001	1.03 (1.01-1.07)	.03
HER2DX pCR score groups						
Low	31	29.0	1 [Reference]	NA	NA	NA
Medium	22	63.6	4.30 (1.34-14.36)	.01	NA	NA
High	27	92.6	30.60 (1.30-156.90)	<.001	NA	NA
HER2DX *ERBB2* score (continuous variable)	80	NA	1.05 (1.02-1.08)	<.001	1.03 (1.00-1.07)	.04
HER2DX *ERBB2* mRNA score						
Low	9	44.4	1 [Reference]	NA	NA	NA
Medium	12	16.7	0.25 (0.03-1.86)	.18		
High	59	71.2	3.09 (0.74-12-91)	.12		
Clinical tumor stage						
cT1	15	80.0	1 [Reference]	NA	NA	NA
cT2-3	65	55.4	0.31 (0.08-1.20)	.09		
Clinical nodal stage						
cN-negative	52	59.6	1 [Reference]	NA	NA	NA
cN-positive	28	60.7	1.05 (0.41-2.68)	.92		
PAM50						
Non-*ERBB2*-enriched	34	35.3	1 [Reference]	NA	1 [Reference]	NA
ERBB2-enriched	46	78.3	6.6 (2.45-17.81)	<.001	2.05 (0.57-7.36)	.27
ERBB2 IHC status						
2+	10	30.0	1 [Reference]	NA	NA	NA
3+	68	66.2	4.57 (1.08-19.32)	.039	NA	NA
Hormone receptor status						
Positive[a]	56	48.2	1 [Reference]	NA	1 [Reference]	NA
Negative	24	87.5	7.52 (2.01-28.10)	.003	1.79 (0.28-12.39)	.54

Abbreviations: IHC, immunohistochemistry; mRNA, messenger RNA; NA, not applicable; OR, odds ratio; PAM50, prediction analysis of microarray 50; pCR, pathologic complete response; THP, paclitaxel/trastuzumab/pertuzumab.

[a] Hormone receptor–positive status was defined as estrogen receptor or progesterone receptor staining of 1% or greater in accordance with current guidelines from American Society of Clinical Oncology/College of American Pathologists.

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 deescalation trial designs can potentially further define the role for this assay in managing early-stage *ERBB2*$^+$ breast cancer.

ARTICLE INFORMATION

Accepted for Publication: December 22, 2022.

Published Online: April 27, 2023. doi:10.1001/jamaoncol.2023.0181

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Acquisition, analysis, or interpretation of data: Waks, Ogayo, Paré, Marín-Aguilera, Brasó-Maristany, Galvan, Castillo, Martínez-Sáez, Vivancos, Villagrasa, Villacampa, Desai, Guerriero, Metzger-Filho, Tung, Parker, Perou, Prat, Tolaney, Mittendorf.
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Obtained funding: Waks, Villagrasa, Prat.
Administrative, technical, or material support: Waks, Marín-Aguilera, Brasó-Maristany, Galvan, Castillo, Guerriero, Metzger-Filho, Tung, Perou.
Supervision: Waks, Martínez-Sáez, Tarantino, Guerriero, Parker, Prat, Tolaney, Mittendorf.
Other - sample processing and banking: Ogayo.

Conflict of Interest Disclosures: Dr Waks reported nonfinancial support from Reveal Genomics during the conduct of the study as well as research support from Genentech, Macrogenics, and Merck and personal fees from AstraZeneca outside the submitted work. Dr Paré reported a patent pending (906 439) and being an employee of Reveal Genomics. Dr Marín-Aguilera reported being an employee of Reveal Genomics. Dr Brasó-Maristany reported a patent for a HER2DX assay pending. Dr Villagrasa reported a patent for EP21383165 issued as well as being a cofounder of Reveal Genomics. Dr Villacampa reported personal fees from MSD, AstraZeneca, Pierre Fabrer, and GSK outside the submitted work. Dr Tarantino reported personal fees from AstraZeneca, Daiichi Sankyo, and Lilly outside the submitted work. Dr Guerriero reported personal fees from Array BioPharma, AstraZeneca, BD Biosciences, Carisma, Codagenix, Duke Street Bio, GlaxoSmithKline, and OncoOne and grants from Array BioPharma, Eli Lilly, Merck, and GlaxoSmithKline outside the submitted work. Dr Metzger-Filho reported grants from Roche/Genentech during the conduct of the study as well as personal fees from Astra Zeneca, Alliance Foundation Trials, Resilience, Oncoclinicas, and Merck and grants from Pfizer outside the submitted work. Dr Tung reported research support and consulting fees from AstraZeneca outside the submitted work. Dr Krop reported grants from Roche during the conduct of the study as well as personal fees from AstraZeneca, Daiichi Sankyo, Genentech/Roche, Macrogenics, Seattle Genetics, Novartis, and Merck and grants from Pfizer outside the submitted work. Dr Parker reported being an equity holder in and consultant for Reveal Genomics during the conduct of the study as well as personal fees from GeneCentric and BMS and a patent with Veracyte outside the submitted work. Dr Perou reported being an equity stockholder in and consultant for Reveal Genomics as well as holding a patent with Bioclassifier outside the submitted work. Dr Prat reported personal fees from Reveal Genomics and Roche during the conduct of the study as well as personal fees from Novartis, AstraZeneca, Daiichi-Sankyo, and Oncolytics Biotech and patents licensed to Reveal Genomics outside the submitted work. Dr Tolaney reported personal fees from Novartis, Pfizer, Merck, Eli Lilly, AstraZeneca, Genentech/Roche, Eisai, Sanofi, Bristol Myers Squibb, Seattle Genetics, Odonate Therapeutics, CytomX Therapeutics, Daiichi Sankyo, Athenex, Gilead, Mersana, Certara, Ellipses Pharma, 4D Pharma, OncoSec Medical Inc, BeyondSpring Pharmaceuticals, OncXerna, Zymeworks, Zentalis, Blueprint Medicines, Reveal Genomics, ARC Therapeutics, Infinity Therapeutics, Chugai Pharmaceuticals, Zetagen, Myovant, Umoja Biopharma, Menarini/Stemline, Bayer, and Aadi Bio as well as grants from Genentech/Roche, Merck, Exelixis, Pfizer, Lilly, Novartis, Bristol Myers Squibb, Eisai, AstraZeneca, NanoString Technologies, Cyclacel, Nektar, Gilead, Sanofi, and Seattle Genetics outside the submitted work. Dr Mittendorf reported service on scientific advisory boards for Astra Zeneca, Exact Sciences, Roche/Genentech, and Merck; steering committee service for BMS, Lilly, and Roche/Genentech; and research support from Roche/Genentech and Gilead during the conduct of the study as well as research funding from Susan Komen for the Cure and participation as a member of the American Society of Clinical Oncology board of directors outside the submitted work. No other disclosures were reported.

Funding/Support: The DAPHNe trial and its correlative analyses were funded by grants from the Breast Cancer Research Foundation (Drs Winer and Waks); Susan G. Komen (Dr Mittendorf), Conquer Cancer Foundation of the American Society of Clinical Oncology (Dr Waks), and Terri Brodeur Breast Cancer Foundation (Dr Waks).

Role of the Funder/Sponsor: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 2.

Meeting Presentation: These data were presented in part at the San Antonio Breast Cancer Symposium; December 6, 2022; San Antonio, Texas.

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