JAMA Oncology | Brief Report

Assessment of a Genomic Assay in Patients With *ERBB2*-Positive Breast Cancer Following Neoadjuvant Trastuzumab-Based Chemotherapy With or Without Pertuzumab

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IMPORTANCE Biomarkers to guide the use of pertuzumab in the treatment of early-stage *ERBB2* (formerly *HER2*)-positive breast cancer beyond simple *ERBB2* status are needed.

OBJECTIVE To determine if use of the HER2DX genomic assay (Reveal Genomics) in pretreatment baseline tissue samples of patients with *ERBB2*-positive breast cancer is associated with response to neoadjuvant trastuzumab-based chemotherapy with or without pertuzumab.

DESIGN, SETTING, AND PARTICIPANTS This is a retrospective diagnostic/prognostic analysis of a multicenter academic observational study in Spain performed during 2018 to 2022 (GOM-HGUGM-2018-05). In addition, a combined analysis with 2 previously reported trials of neoadjuvant cohorts with results from the assay (DAPHNe and I-SPY2) was performed. All patients had stage I to III *ERBB2*-positive breast cancer, signed informed consent, and had available formalin-fixed paraffin-embedded tumor specimens obtained prior to starting therapy.

EXPOSURES Patients received intravenous trastuzumab, 8 mg/kg, loading dose, followed by 6 mg/kg every 3 weeks in combination with intravenous docetaxel, 75 mg/m^2, every 3 weeks and intravenous carboplatin area under the curve of 6 every 3 weeks for 6 cycles, or this regimen plus intravenous pertuzumab, 840 mg, loading dose, followed by an intravenous 420-mg dose every 3 weeks for 6 cycles.

MAIN OUTCOME AND MEASURES Association of baseline assay-reported pathologic complete response (pCR) score with pCR in the breast and axilla, as well as association of baseline assay-reported pCR score with response to pertuzumab.

RESULTS The assay was evaluated in 155 patients with *ERBB2*-positive breast cancer (mean [range] age, 50.3 [26-78] years). Clinical T1 to T2 and node-positive disease was present in 113 (72.9%) and 99 (63.9%) patients, respectively, and 105 (67.7%) tumors were hormone receptor positive. The overall pCR rate was 57.4% (95% CI, 49.2%-65.2%). The proportion of patients in the assay-reported pCR-low, pCR-medium, and pCR-high groups was 53 (34.2%), 54 (34.8%), and 48 (31.0%), respectively. In the multivariable analysis, the assay-reported pCR score (as a continuous variable from 0-100) showed a statistically significant association with pCR (odds ratio [OR] per 10-unit increase, 1.43; 95% CI, 1.22-1.70; $P < .001$). The pCR rates in the assay-reported pCR-high and pCR-low groups were 75.0% and 28.3%, respectively (OR, 7.85; 95% CI, 2.67-24.91; $P < .001$). In the combined analysis (n = 282), an increase in pCR rate due to pertuzumab was found in the assay-reported pCR-high tumors (OR, 5.36; 95% CI, 1.89-15.20; $P < .001$) but not in the assay-reported pCR-low tumors (OR, 0.86; 95% CI, 0.30-2.46; $P = .77$). A statistically significant interaction between the assay-reported pCR score and the effect of pertuzumab in pCR was observed.

CONCLUSIONS AND RELEVANCE This diagnostic/prognostic study demonstrated that the genomic assay predicted pCR following neoadjuvant trastuzumab-based chemotherapy with or without pertuzumab. This assay could guide therapeutic decisions regarding the use of neoadjuvant pertuzumab.

JAMA Oncol. doi:10.1001/jamaoncol.2023.0187
Published online April 27, 2023.

Related article

Supplemental content

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Pertuzumab is approved for treatment of early and advanced *ERBB2* (formerly *HER2*)-positive breast cancer.[1,2] In early disease, the addition of pertuzumab to trastuzumab-based chemotherapy increases pathologic complete response (pCR) rates.[3] In the NeoSphere phase 2 trial,[3] pertuzumab and trastuzumab plus docetaxel showed an improvement in the pCR rate compared with trastuzumab plus docetaxel (Δ, 16.8%). Moreover, the addition of 1 year of pertuzumab to trastuzumab-based chemotherapy improved invasive disease–free survival (6-year survival, 91% vs 88%).[4,5] Of note, the benefit was restricted to node-positive disease, and no overall survival benefit was observed.[5] Overall, the benefits of pertuzumab in early-stage *ERBB2*-positive disease are modest.

HER2DX (Reveal Genomics) is a clinically available genomic test that provides 2 scores to predict long-term prognosis (ie, risk score) and likelihood of pCR (ie, pCR score) in early *ERBB2*-positive breast cancer.[6] The 27-gene assay integrates clinical and biological information tracking immune response, luminal differentiation, tumor proliferation, and expression of the *ERBB2* amplicon.[6] This diagnostic/prognostic study aims to determine if use of this genomic assay in pretreatment baseline tissue samples is associated with response to neoadjuvant trastuzumab-based chemotherapy with or without pertuzumab.

Key Points

Question Can the HER2DX genomic assay (Reveal Genomics) predict response to neoadjuvant trastuzumab-based chemotherapy with or without pertuzumab in early-stage *ERBB2*-positive breast cancer?

Findings In this diagnostic study of 155 patients with *ERBB2* (formerly *HER2*)-positive breast cancer, the assay-reported pathologic complete response (pCR) score showed statistically significant association with pCR following trastuzumab-based chemotherapy independently of pertuzumab use. More importantly, a statistically significant increase in pCR rates with the addition of pertuzumab was only observed in assay-reported pCR-high disease, which represented 1 of 3 patients with *ERBB2*-positive breast cancer.

Meaning This assay might provide meaningful clinical information to guide therapeutic decisions regarding the use of trastuzumab-based chemotherapy with or without pertuzumab in the neoadjuvant setting.

Methods

GOM-HGUGM-2018-05 Cohort

GOM-HGUGM-2018-05 (hereafter, GOM) is a prospective observational study of consecutive patients with stage I to III *ERBB2*-positive breast cancer treated with neoadjuvant therapy across 7 hospitals in Spain. Patients received 6 cycles of intravenous docetaxel, 75 mg/m^2, every 3 weeks in combination with intravenous carboplatin area under the curve of 6 every 3 weeks and intravenous trastuzumab, 8 mg/kg, loading dose followed by 6 mg/kg every 3 weeks (TCH). Once neoadjuvant pertuzumab was reimbursed in Spain, most patients received TCH in combination with intravenous pertuzumab, 840 mg, loading dose, followed by an intravenous 420-mg dose every 3 weeks (TCHP).

This study followed the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guideline[7] and was approved by an ethics committee at Hospital General Universitario Gregorio Marañón. Patients signed written informed consent.

DAPHNe and I-SPY2 Cohorts

Ninety-eight patients in DAPHNe, a prospective single-arm phase 2 study, were treated with preoperative paclitaxel, 80 mg/m^2, weekly for 12 weeks in combination with trastuzumab and pertuzumab.[8] The HER2DX results in DAPHNe are reported elsewhere.[9]

The I-SPY2 study[10] adaptively randomized 128 patients with stage II to III *ERBB2*-positive breast cancer to 4 cycles of intravenous T-DM1, 3.6 mg/kg, every 3 weeks in combination with pertuzumab (n = 52); paclitaxel, trastuzumab, and pertuzumab (n = 45); or a control arm of paclitaxel and trastuzumab (n = 31). Patients received 4 cycles of doxorubicin, 60 mg/m^2, and cyclophosphamide, 600 mg/m^2, intravenously, every 2 to 3 weeks, before surgery. The primary results of HER2DX in I-SPY2 have been reported elsewhere.[6]

HER2DX

In the GOM and DAPHNe cohorts, the HER2DX standardized assay was performed from pretreatment baseline samples, as previously described.[6] Preestablished cutoffs were used for each score. In I-SPY2, HER2DX was applied onto publicly available microarray data (GSE181574) from 127 patients,[10] as previously described.[6]

Statistical Analysis

The primary objective was to evaluate the association between the HER2DX-reported pCR score and pCR in the breast and axilla. Univariable and multivariable logistic regression models were used. To build the multivariable model, the least absolute shrinkage and selection operator regression was used for variable selection. The C statistic was calculated to determine the discrimination capacity of HER2DX.

The secondary objective was to evaluate the ability of HER2DX to predict response to neoadjuvant pertuzumab. To accomplish this goal, a combined patient-level analysis of 3 cohorts (GOM, DAPHNe, I-SPY2) was undertaken. Pooled odds ratios (ORs) and 95% CIs were calculated with random effect models using the DerSimonian-Laird method, and the I^2 was reported to estimate the percentage of total variability due to between-cohort heterogeneity. Interaction tests, used to evaluate the different pertuzumab effect according to HER2DX-reported pCR groups, were adjusted by cohort. Across the 3 cohorts, pCR was defined as ypT0/isN0. The significance level was set to a 2-sided $\alpha = .05$. Statistical computations were carried out in R, version 4.0.3 (R Foundation for Statistical Computing).

Results

GOM Cohort Characteristics

As of June 2022, 155 patients with available pretreatment baseline RNA had enrolled in the study (**Table 1** and eTable 1 and eFigure 1 in Supplement 1). Briefly, the mean (range) age of patients was 50.3 (26-78) years, and 85 patients (55.2%) were premenopausal. Clinical T1 to T2 disease was present in 113 (72.9%) patients, clinical node-positive disease (cN1-cN3) was present in 99 (63.9%) patients, and 105 (67.7%) tumors were hormone receptor positive. Sixty-seven (43.2%) and 88 (56.8%) patients received TCH and TCHP, respectively. The overall pCR rate was 57.4% (95% CI, 49.2%-65.2%): 52.2% (95% CI, 39.8%-64.4%) among those receiving TCH and 61.4% (95% CI, 50.3%-71.4%) among those receiving TCHP.

Assay-Reported pCR Score in the GOM Cohort

The assay-reported pCR score (range, 0-100) showed a statistically significant association with pCR (OR per 10-unit increase, 1.43; 95% CI, 1.22-1.70; $P < .001$) after adjusting for treatment and clinicopathological factors (**Table 2**). The pCR rates in the assay-reported pCR-high and pCR-low groups were 75.0% and 28.3%, respectively (OR, 7.85; 95% CI, 2.67-24.91; $P < .001$). In patients treated with TCHP, the pCR rates in the assay-reported pCR-high and pCR-low groups were 85.7% and 27.3%, respectively (OR, 16.0; 95% CI, 4.72-67.09; $P < .001$). The C statistics for the assay-reported pCR score (as a continuous variable) were 0.746 (all population) and 0.812 (TCHP).

Assay-Reported pCR Score and Pertuzumab Response

A total of 264 (72.9%) and 98 (27.1%) patients received and did not receive neoadjuvant pertuzumab, respectively (eTables 1-3 in Supplement 1). No statistically significant difference in pCR rates was found across the 3 studies. The overall pCR rates in patients treated with and without pertuzumab were 59.8% and

Table 1. Patient Characteristics Among the GOM Neoadjuvant Cohort (N = 155)

Characteristic	No. (%)
Pathological response in breast and axilla	
Complete response	89 (57.4)
Residual disease	66 (42.6)
Hormone receptor status	
Positive	105 (67.7)
Negative	50 (32.3)
Intrinsic subtype	
Luminal A	38 (24.5)
Luminal B	26 (16.8)
ERBB2 enriched	80 (51.6)
Basallike	8 (5.2)
Normallike	3 (1.9)

Abbreviation: GOM, GOM-HGUGM-2018-05 trial.

Table 2. Association of Pretreatment Baseline Variables With Response in 155 Patients With *ERBB2*-Positive Early-Stage Breast Cancer Treated With Neoadjuvant TCH or TCHP in the GOM-HGUGM-2018-05 Cohort

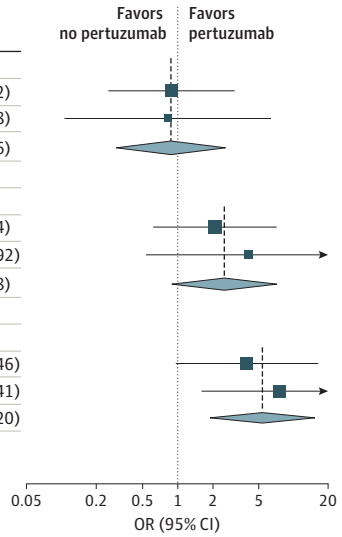
			Univariate model		Multivariable model	
Characteristic	Patients, No.	pCR rate	OR (95% CI)	*P* value	OR (95% CI)	*P* value
HER2DX pCR score (10-unit increase)	155	NA	1.39 (1.23-1.60)	<.001	1.43 (1.22-1.70)	<.001
HER2DX pCR score groups						
Low	53	28.3%	1 [Reference]	NA	1 [Reference]	NA[a]
Medium	54	70.4%	6.02 (2.67-14.27)	<.001	6.58 (2.50-18.75)	<.001[a]
High	48	75.0%	7.60 (3.22-19.09)	<.001	7.85 (2.67-24.91)	<.001[a]
Clinical tumor stage						
cT1-cT2	113	60.2%	1 [Reference]	NA	NA	NA
cT3-cT4	42	50.0%	0.66 (0.32-1.35)	.26	NA	NA
Clinical nodal stage						
cN0	56	69.6%	1 [Reference]	NA	1 [Reference]	NA
cN1-cN3	99	50.5%	0.44 (0.22-0.88)	.02	0.36 (0.15-0.81)	.02
PAM50						
ERBB2 enriched	80	68.8%	1 [Reference]	NA	1 [Reference]	NA
Non-*ERBB2* enriched	75	45.3%	0.38 (0.19-0.72)	.004	0.65 (0.26-1.62)	.35
Treatment						
TCH	67	52.2%	1 [Reference]	NA	1 [Reference]	NA
TCHP	88	61.4%	1.45 (0.76-2.77)	.26	1.97 (0.90-4.44)	.09
Hormone receptor status						
Positive	105	51.4%	1 [Reference]	NA	NA	NA
Negative	50	70.0%	2.20 (1.09-4.60)	.03	NA	NA
Age (10-unit increase)	155	NA	0.71 (0.51-0.98)	.04	0.70 (0.47-1.01)	.07
Ki-67 IHC (10-unit increase)	155	NA	1.04 (0.89-1.23)	.60	0.82 (0.66-1.01)	.07

Abbreviations: IHC, immunohistochemistry; NA, not applicable; OR, odds ratio; pCR, pathologic complete response; TCH, docetaxel, carboplatin, trastuzumab; TCHP, docetaxel, carboplatin, trastuzumab, pertuzumab.

[a] A separate multivariable model has been performed using HER2DX-reported pCR score groups instead of HER2DX-reported pCR score. To avoid multicollinearity, HER2DX-reported pCR score groups and HER2DX-reported pCR score cannot be included in the same model.

Figure. Association of HER2DX Pathologic Complete Response (pCR) Groups With Response to Pertuzumab in a Combined Patient-Level Analysis (N = 282)

Group	Patients, No.	OR (95% CI)
Low		
GOM	53	0.88 (0.25-3.02)
I-SPY2	42	0.81 (0.11-6.08)
Overall	95	0.86 (0.30-2.46)
Heterogeneity: I^2 = 0%, P = .95		
Medium		
GOM	54	2.06 (0.61-6.94)
I-SPY2	42	4.05 (0.53-30.92)
Overall	96	2.46 (0.87-6.98)
Heterogeneity: I^2 = 0%, P = .58		
High		
GOM	48	4.00 (0.97-16.46)
I-SPY2	43	7.59 (1.63-35.41)
Overall	91	5.36 (1.89-15.20)
Heterogeneity: I^2 = 0%, P = .55		



Data are from the neoadjuvant cohorts in the GOM-HGUGM-2018-05 (GOM) and I-SPY2 trials. OR indicates odds ratio.

43.9%, respectively (Δ, 15.9%; OR, 2.09; 95% CI, 1.26-3.52; P = .005; eFigure 2 in Supplement 1). The pCR rates with and without pertuzumab differed according to assay-reported pCR score (eFigure 2 in Supplement 1). In patients with assay-reported pCR-high, pCR-medium, and pCR-low disease, the difference in pCR rates (with pertuzumab vs without pertuzumab) were 34.4%, 12.7%, and 0.2% in favor of pertuzumab, respectively.

In the combined patient-level analysis of GOM and I-SPY2 cohorts (**Figure** and eFigures 2 and 3 in Supplement 1), an increase in pCR rate associated with pertuzumab was found in assay-reported pCR-high tumors (OR, 5.36; 95% CI, 1.89-15.20; P < .001) but not in assay-reported pCR-low tumors (OR, 0.86; 95% CI, 0.30-2.46; P = .77). A statistically significant interaction was observed between the assay-reported pCR-high group vs pCR-medium and pCR-low groups, and the pCR-high group vs the pCR-low group (eFigure 2 in Supplement 1).

Discussion

To our knowledge, this is the first study to demonstrate that the HER2DX-reported pCR score predicts response to neoadjuvant pertuzumab. A potential biological explanation is that the assay-reported pCR-high disease is composed of *ERBB2*-positive tumors that have the highest expression and activity of *ERBB2* and/or the highest infiltration of B and T immune cells,[6] all of which are biological features previously associated with pertuzumab response.[11,12] In contrast, the pCR rate in the assay-reported pCR-low disease is low (ie, <25%) and does not increase with pertuzumab. Less clear is the value of pertuzumab in the assay-reported pCR-medium group.

Pertuzumab is approved for treatment of clinically high-risk *ERBB2*-positive breast cancer. However, the absolute increase in pCR rates in unselected patients with stage II to III disease in the NeoSphere trial is less than 20%.[3] In addition, the absolute increase in invasive disease–free survival when 1 year of pertuzumab is added to trastuzumab-based chemotherapy is small, except in node-positive disease (Δ, 4.9% at 8 years).[4,5] These modest results from the NeoSphere and APHINITY trials have led many countries to decline reimbursement of pertuzumab in early-stage disease or to limit its use in the adjuvant setting if the cancer is node positive. Thus, a biomarker such as the present genomic assay, which can help identify patients who will benefit the most from neoadjuvant pertuzumab, might be of clinical value.

Limitations

The retrospective nature of this study and the lack of randomization and long-term survival outcomes represent the main limitations. Another limitation is that the assay was evaluated in silico in the I-SPY2 cohort and that we did not address if the type of chemotherapy backbone mattered.

Conclusions

This diagnostic/prognostic study showed that the HER2DX genomic assay can predict pCR following neoadjuvant trastuzumab-based chemotherapy with or without pertuzumab. This assay could guide therapeutic decisions regarding the use of neoadjuvant pertuzumab.

ARTICLE INFORMATION

Accepted for Publication: December 22, 2022.

Published Online: April 27, 2023. doi:10.1001/jamaoncol.2023.0187

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Obtained funding: Waks, Villagrasa, Prat.
Administrative, technical, or material support: Echavarría, López-Tarruella, Roche-Molina, del Monte-Millán, Ayala de la Peña, Rodríguez-Lescure, Marín-Aguilera, Galván, Brasó-Maristany, Waks, Tolaney, Perou, Martín.
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Conflict of Interest Disclosures: Dr Bueno-Muiño reported grants from Novartis, AstraZeneca, Lilly, Roche, and Pfizer; speaker honoraria from Roche, Novartis, Lilly, MSD, AstraZeneca, and GSK; and congress attendance for Pfizer and GSK outside the submitted work. Dr Echavarría reported speaker fees from Roche, Teva, Novartis, Pfizer, and Lilly, as well as serving in advisory roles for Lilly and AstraZeneca. Dr Lopez-Tarruella reported personal fees from AstraZeneca, Novartis, Roche, Pfizer, Pierre Fabre, Lilly, Seagen, Europe GmbH, Daiichi Sankyo, Gilead, GSK, MSD, and Veracyte, as well as speaker fees from Lilly outside the submitted work. Dr Massarrah reported travel grants from Novartis and AstraZeneca, as well as personal fees from AstraZeneca, Novartis, Roche, and GSK. Dr Jerez reported travel and training grants from Roche, Novartis, Pfizer, and Lilly; personal fees from Novartis, Pfizer, Roche, and AstraZeneca; and speaker honoraria from Roche, Novartis, Lilly, and AstraZeneca. Dr Ayala de la Peña reported personal fees from Roche, Sanofi, Lilly, AstraZeneca, Daiichi Sankyo, Pfizer, Seagen, and MSD outside the submitted work. Dr García-Sáenz reported grants from AstraZeneca, Daiichi Sankyo, Lilly, Novartis, Gilead, Seagen, and Exact Sciences outside the submitted work; personal fees from Seagen, Gilead, Sanofi, Novartis, Celgene, Lilly, Eisai, AstraZeneca, Daiichi Sankyo, MSD, and Pierre Fabre; institution research funding from AstraZeneca; and travel support from Novartis, Roche, and Pfizer. Dr Moreno reported grants from Pfizer and personal fees from Pfizer, Novartis, Roche, MSD, AstraZeneca, Daiichi Sankyo, Gilead, and Exact Sciences outside the submitted work. Dr Rodríguez-Lescure reported personal fees from Roche during the conduct of the study and personal fees from AstraZeneca, Pfizer, Novartis, Lilly, Seagen, Pierre Fabre, Daiichi Sankyo, MSD, and Gilead outside the submitted work. Dr Ballesteros García reported personal fees from Pfizer, Novartis, Seagen, Roche, and Pierre Fabre. Dr Brasó-Maristany reported a patent pending for HER2DX. Dr Waks reported support for the HER2DX assay from Reveal Genomics during the conduct of the study, as well as institutional research support from Genentech, MacroGenics, and Merck outside the submitted work. Dr Tolaney reported personal fees from Novartis, Pfizer, Merck, Lilly, Nektar, NanoString Technologies, Puma Biotechnology, 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, BeyondSpring Pharmaceuticals, OncXerna, Zymeworks, Zentalis, Blueprint Medicines, Reveal Genomics, ARC Therapeutics, Infinity Therapeutics, Chugai Pharmaceuticals, Myovant, Zetagen, Umoja Biopharma, Menarini/Stemline, Aadi Bio, and Bayer, as well as institutional research 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 compensated service on scientific advisory boards for AstraZeneca, Exact Sciences, Merck, and Roche/Genentech; uncompensated service on steering committees for Bristol Myers Squibb, Lilly, and Roche/Genentech; and institutional research support from Roche/Genentech (via a Stand Up To Cancer grant) and Gilead, all during the conduct of the study; she also reported research funding from Susan G. Komen for the Cure, for which she serves as a scientific advisor, and uncompensated participation as a member of the board of directors for the American Society of Clinical Oncology. Dr Villagrasa reported a patent issued for EP21383165.4. Prof Parker reported being an equity stockholder in and consultant for Reveal Genomics during the conduct of the study; personal fees from GeneCentric and Bristol Myers Squibb outside the submitted work; and listed as an inventor on the patent applications for the Breast PAM50 assay with royalties paid from Veracyte as well as the patent application on DNA-based predictors of breast tumor phenotypes. Prof Perou reported being an equity stockholder in and consultant for Bioclassifier LLC and Reveal Genomics outside the submitted work; a patent for US Patent No. 12,995,459 with royalties paid from Bioclassifier LLC; and listed as an inventor on patent applications for the Breast PAM50 assay. Dr Paré reported being listed as an inventor on patent PCT/EP2021/070788. Dr Villacampa reported personal fees from GSK, AstraZeneca, Pierre Fabre, and MSD outside the submitted work. Prof Prat reported personal fees from Reveal Genomics and Roche during the conduct of the study; personal fees from Novartis, Oncolytics Biotech, Daiichi Sankyo, Pfizer, Amgen, Bristol Myers Squibb, Puma, MSD, Guardant Health, Peptomyc, Lilly, Roche, NanoString Technologies, and AstraZeneca outside the submitted work; institutional financial interests from Boehringer Ingelheim, Novartis, Roche, NanoString Technologies, Sysmex Europe GmbH, Medica Scientia Innovation Research, Celgene, Astellas, and Pfizer; being stockholder in and consultant for Reveal Genomics; and a patent for HER2DX licensed to Reveal Genomics as well as a patent for ERBB2 issued Reveal Genomics. Dr Martín reported research grants from Puma; personal fees from Roche, Novartis, AstraZeneca, Daiichi Sankyo, Pfizer, Lilly, Sanofi, and Seagen outside the submitted work; and serving as chairman for GEICAM and a member of the board of directors for Trio. No other disclosures were reported.

Funding/Support: This study was funded by Hospital General Universitario Gregorio Marañón in Madrid, Spain, and Reveal Genomics in Barcelona, Spain. Dr Martín received funding from CIBER and the Center of Biomedical Research on Cancer (CIBERONC, group CB16/12/00471). Dr Brasó-Maristany received funding from the Fundación Científica Asociación Española Contra el Cáncer (research grant AECC 2021-INVES21943BRAS). Dr Mittendorf is supported through the Rob and Karen Hale Distinguished Chair in Surgical Oncology at Brigham and Women's Hospital.

Role of the Funder/Sponsor: No funders were involved 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.

Meeting Presentation: This study was presented in part at the annual San Antonio Breast Cancer Symposium; December 7, 2022; San Antonio, Texas.

Data Sharing Statement: See Supplement 2.

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