HER2DX ERBB2 mRNA expression in advanced HER2-positive breast cancer treated with T-DM1

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Keywords: HER2, breast cancer, T-DM1, ERBB2, gene expression, HER2DX, brain metastasis

Abstract

In advanced HER2-positive (HER2+) breast cancer (BC), the new antibody-drug conjugate trastuzumab deruxtecan (T-DXd) is more effective compared to trastuzumab emtansine (T-DM1). However, T-DXd can have significant toxicities, and the right treatment sequence is unknown. Biomarkers to guide the use of anti-HER2 therapies beyond HER2 status are needed. Here, we evaluated if pre-established levels of ERBB2 mRNA expression according to the HER2DX standardized assay are associated with response and survival following T-DM1. In ERBB2 low, medium, and high groups, the overall response rate was 0%, 29% and 56%, respectively (P<.001). ERBB2 mRNA was significantly associated with better progression-free survival (p=0.002) and overall survival (OS; P=0.02). These findings were independent of HER2 IHC levels, hormone receptor, age, brain metastasis and line of therapy. The HER2DX risk-score (P=.04) and the immunoglobulin (IGG) signature (P=.04) were significantly associated with OS since diagnosis. HER2DX provides prognostic and predictive information following T-DM1 in advanced HER2+ BC.

Antibody drug-conjugates (ADCs) targeting HER2 have changed the treatment landscape of HER2-positive (HER2+) advanced breast cancer (BC)[1-5]. Among them, trastuzumab-emtansine (T-DM1) improves progression-free survival (PFS) and overall survival (OS) in patients with HER2+ metastatic BC previously treated with trastuzumab and a taxane[3,5]. Recently, trastuzumab-deruxtecan (T-DXd) has shown superiority to T-DM1 in the 2nd line setting[2]. However, the toxicity profile of T-DXd is not trivial. In addition, T-DXd is highly efficacious after T-DM1[1], but no data exists about the activity of T-DM1 after T-DXd and uncertainty exists regarding the best treatment sequence[6].

To date, no biomarker of prognosis and/or treatment benefit has been implemented in advanced HER2+ BC. In early-stage HER2+ BC, the HER2DX assay is prognostic and predictive[7]. HER2DX provides an ERBB2 mRNA score with specific cutoffs to identify HER2+ from HER2-negative BC according to ASCO/CAP guidelines[8], and two different expression levels within HER2+ BC (medium and high). ERBB2 mRNA might be a potential predictive biomarker of T-DM1 response[9-12]. Here we evaluated the HER2DX variables in patients with advanced HER2+ BC treated with T-DM1 (**Supplementary Methods**).

Eighty-seven consecutive patients diagnosed with HER2+ advanced BC and treated with T-DM1 were evaluated (**Figure 1, A**). Baseline patient characteristics are reported in **Table 1**. Median follow-up since T-DM1 initiation was 35.8 months. Overall response rate (ORR), median PFS and median OS were 45% (6 complete and 33 partial responses), 5.8 and 24.3 months respectively (**Figure 1,B-C**).

ERBB2 mRNA range (5.1-fold difference between lowest and highest quartiles) varied according to centrally reviewed HER2 IHC (**Figure 2, A**). According to pre-established cutoffs, ERBB2 mRNA high, medium, and low groups represented 70.2%, 19.5%

and 10.3%, respectively. ERBB2 mRNA was significantly associated with ORR as a continuous variable (**Figure 2, B**; **Supplementary Table 2**), and according to prespecified cutoffs (odds ratio=5.29, *P*=.003).

High ERBB2 expression was significantly associated with better PFS and OS as a continuous (**Supplementary Table 2**) and as a categorical variable according to prespecified cutoffs (**Figure 2, C-D**). ERBB2 remained significantly associated with ORR and PFS when adjusted by the other clinical-pathological variables (**Supplementary Table 3**). Notably, HER2 IHC was significantly associated with better PFS and OS in univariate analyses but it was not when ERBB2 expression was included in multivariable analyses (**Table 2**). In the patient subset treated with T-DM1 in the 1st-3rd line, ERBB2 was significantly associated with better PFS (HR=0.70, 95% CI=0.58-0.86, *P*<.001) and OS (HR=0.75, 95% CI=0.61-0.92, *P*=.005) as a continuous and as a categorical variable (**Figure 2, E-F**).

To further validate the value of ERBB2 mRNA in advanced HER2+ BC treated with anti-HER2 therapies, we interrogated tumor samples of 91 patients treated with trastuzumab and lapatinib in the EGF104900 phase III trial[13]. ERBB2 mRNA was associated with better PFS (HR=0.81, 95% CI=0.72-0.91, *P*<.001) and OS (HR=0.85, 95% CI=0.75-0.95, *P*=.006) as a continuous variable and as group categories (**Supplementary Figure 1**). Of note, ERBB2-low disease in this study represented 19.8% of all cases.

In the T-DM1 dataset, we also explored the impact of tissue type (primary versus metastasis) in the ability of HER2DX ERBB2 mRNA to predict prognosis. In a univariate analysis, tissue type was not significantly associated with PFS and OS (**Supplementary Table 3**), and did not impact the association of ERBB2 mRNA with PFS (adjusted HR=0.74, 95% CI=0.62-0.88, *P*<.001) and OS (adjusted HR=0.81, 95% CI=0.68-0.96, *P*=.01).

Additionally, we evaluated 24 HER2+ paired primary and metastatic samples of an internal dataset[14], ERBB2 expression did not show a significant difference betweeSupplementaryn tissue types, suggesting that it is overall stable during tumor evolution (**Supplementary Figure 2**).

Finally, we explored other variables provided by the HER2DX assay. The HER2 amplicon signature was significantly associated with ORR, PFS and OS as continuous (**Supplementary Table 1**) and categorical variable (**Supplementary Figure 3, A-C**). HER2 amplicon score and ERBB2 expression were moderately correlated (Pearson coefficient=0.59, *P*<.001). HER2DX pCR score was significantly associated with ORR and OS but not PFS (**Supplementary Table 1**).

HER2DX risk and IGG signature scores, as continuous variables, were significantly associated with OS from diagnosis (OSD) (HR=1.36, 95%CI=1.02-1.83, *P*=.04 and HR=0.73, 95% CI=0.54-0.98, *P*=0.04 respectively). IGG signature as a categorical variable was also associated with OSD (**Supplementary Figure 3, D**). Forty-eight patients had a prior diagnosis of early-stage HER2+ BC, in 21 of these cases HER2DX was performed in the primary tumor, including tumor and nodal staging. HER2DX high-risk disease was identified in 20 (95%) of 21 patients who had a prior diagnosis of early-stage HER2+ BC. The only patient with HER2DX low-risk disease had been diagnosed in 2003 of a pT1cN0 hormone receptor-positive disease and presented distant metastasis in 2014. Finally, we observed a significant association of HER2DX risk-score with OS (HR=1.27, 95% CI=1.05-1.52, *P*=0.01) in 125 patients with HER2+ BC who relapsed at a distant site from the publicly available METABRIC dataset[15].

Here, we show that the standardized HER2DX genomic assay provides potential

predictive and prognostic value in advanced HER2+ BC treated with T-DM1. ERBB2 mRNA had been previously associated with T-DM1 benefit in retrospective analyses of three trials[10,11,12] in HER2+ metastatic BC. However, these research-based determinations of ERBB2 mRNA and did not evaluate specific cutoffs.

The HER2DX ERBB2-low group, which represents 10-20% of HER2+ tumors, has an extremely poor response to T-DM1 and survival outcome. This group might benefit from other ADCs such as T-DXd[2], and might be spared T-DM1, reducing unnecessary toxicities and relatively high costs. In contrast, HER2DX ERBB2 high-group might be good candidates to indicate T-DM1 because of: high efficacy of T-DM1, lower cost than T-DXd, less toxicity than T-DXd and high efficacy of T-DXd at progression from T-DM1[1]. In contrast, no data exist regarding the activity of T-DM1 after progression to T-DXd.

Limitations of this study are the retrospective nature design, along with the limited number of patients involved. Patients were treated according to everyday clinical practice and were heterogeneous with respect to the previous treatments received, and tissue type available.

To conclude, the introduction of new anti-HER2 drugs is changing the treatment landscape and improving outcomes with median OS exceeding 5-years. Questions remain unanswered regarding the optimal therapies and sequencing strategies for each patient. To guide these decisions, implementation of prognostic and predictive biomarkers will be needed.

Funding

This study was funded by Hospital Clinic, DiSCOG – University of Padova DOR 1721185/17

and DOR 1830512/18 (to MV.D.), and Reveal Genomics (Barcelona, Spain). F. B-M received funding from Fundación Científica Asociación Española Contra el Cáncer (Ayudas Investigador AECC 2021- INVES21943BRAS). F.S. received funding from the European Society for Medical Oncology (ESMO Fellowship – Translational) and BBVA Foundation/Hospital Clinic of Barcelona (Joan Rodés - Jose Baselga Advanced Research Contract in Oncology).

Notes

Role of the Funder: The funders, except for ESMO, BBVA Foundation and AECC, had a 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.

Disclosures: Potential conflicts of interest are the following: A.P. reports advisory and consulting fees from Roche, Pfizer, Novartis, Amgen, BMS, Puma, Oncolytics Biotech, MSD, Guardan Health, Peptomyc and Lilly, lecture fees from Roche, Pfizer, Novartis, Amgen, BMS, Nanostring Technologies and Daiichi Sankyo, institutional financial interests from Boehringer, Novartis, Roche, Nanostring, Sysmex Europa GmbH, Medica Scientia Innovation Research, SL, Celgene, Astellas and Pfizer; stockholder and hold a salary from Reveal Genomics, SL and patents from HER2DX. C.M.P is an equity stockholder and consultant of BioClassifier LLC, and for Reveal Genomics. C.M.P is also listed as an inventor on patent applications for the Breast PAM50 assay. J.S.P is an equity stockholder and consultant for Reveal Genomics and is also listed as an inventor on patent applications for the Breast PAM50 assay, and a patent application on DNA-based predictors of breast tumor

phenotypes. F.B-M. has a patent application EP21383165. L.P is listed as an inventor on patent PCT/EP2021/070788. G.G reports fees for invited speaker from EliLilly and Novartis, and Consulting Fees from Gilead. M-V.D reports fees for Consulting Fees and Fees for Non-CME Services from Astrazeneca. Daiichi Sankyo, EliLilly, Exact Sciences, Gilead, MSD, Novartis, Pfizer, Seagen. FM reports consulting fees from Novartis and Roche. V.G. reports Consulting Fees and Fees for Non-CME Services from Amgen, Exact Science, Gilead, GSK, Lilly, MerkSerono, MSD, Pfizer and Sanofi.

Author contributions: Conceptualization: AP, AV, CMP and JSP. Data curation: FBM, GG, NC, TP, MVD, FM, TG, OMS, MMA, FS, BC, LA, MV, BA and MM. Formal Analysis: AP, FBM, GG. Methodology: AP, FBM, GG, LP, PG, ES and BG. Supervision: AV, CMP, JSP, PFC, AP, VG. Writing - original draft: AP and FBM. Writing - review & editing: FBM, GG, NC, TP, LP, JM, PG, MVD, FM, TG, OMS, MMA, FS, BC, LA, MV, BA, MM, ES, BG, AV, PV, JSP, CMP, PFC, AP, VG.

Data Availability

Due to the nature of this research, participants of this study did not agree for their data to be shared publicly. However, data can be made available under a data transfer agreement and upon Ethics Committee approval and we encourage investigators interested in data access and collaboration to request them using the following link: https://www.clinicbarcelona.org/en/idibaps/research-areas/oncology-and-haematology/translational-genomics-and-targeted-therapies-in-solid-tumours/tools

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Figure Legends

Figure 1. Study design. (**A**) HER2DX standardized assay was performed in archival FFPE tumor biopsies from patients with advanced HER2+ BC treated with T-DM1. HER2DX was evaluated in archival FFPE tumor samples. (**B**) Progression-free survival (PFS) in all patients. (**C**) Overall survival (OS) in all patients.

Figure 2. Association of ERBB2 mRNA expression with efficacy following T-DM1. (**A**) ERBB2 mRNA expression across the centrally reviewed HER2 IHC groups. (**B**) ERBB2 mRNA expression in patients with stable disease (SD)/progressive disease (PD) versus partial or complete response (PR/CR). (**C**) PFS according to ERBB2 mRNA expression (pre-established cutoffs). (**D**) OS according to ERBB2 mRNA expression (pre-established cutoffs). (**E**) PFS according to ERBB2 mRNA expression (pre-established cutoffs) in patients treated with TDM1 in the 1st to 3rd line setting. (**F**) OS according to ERBB2 mRNA expression (pre-established cutoffs) in patients treated with TDM1 in the 1st to 3rd line setting.

Figure 1

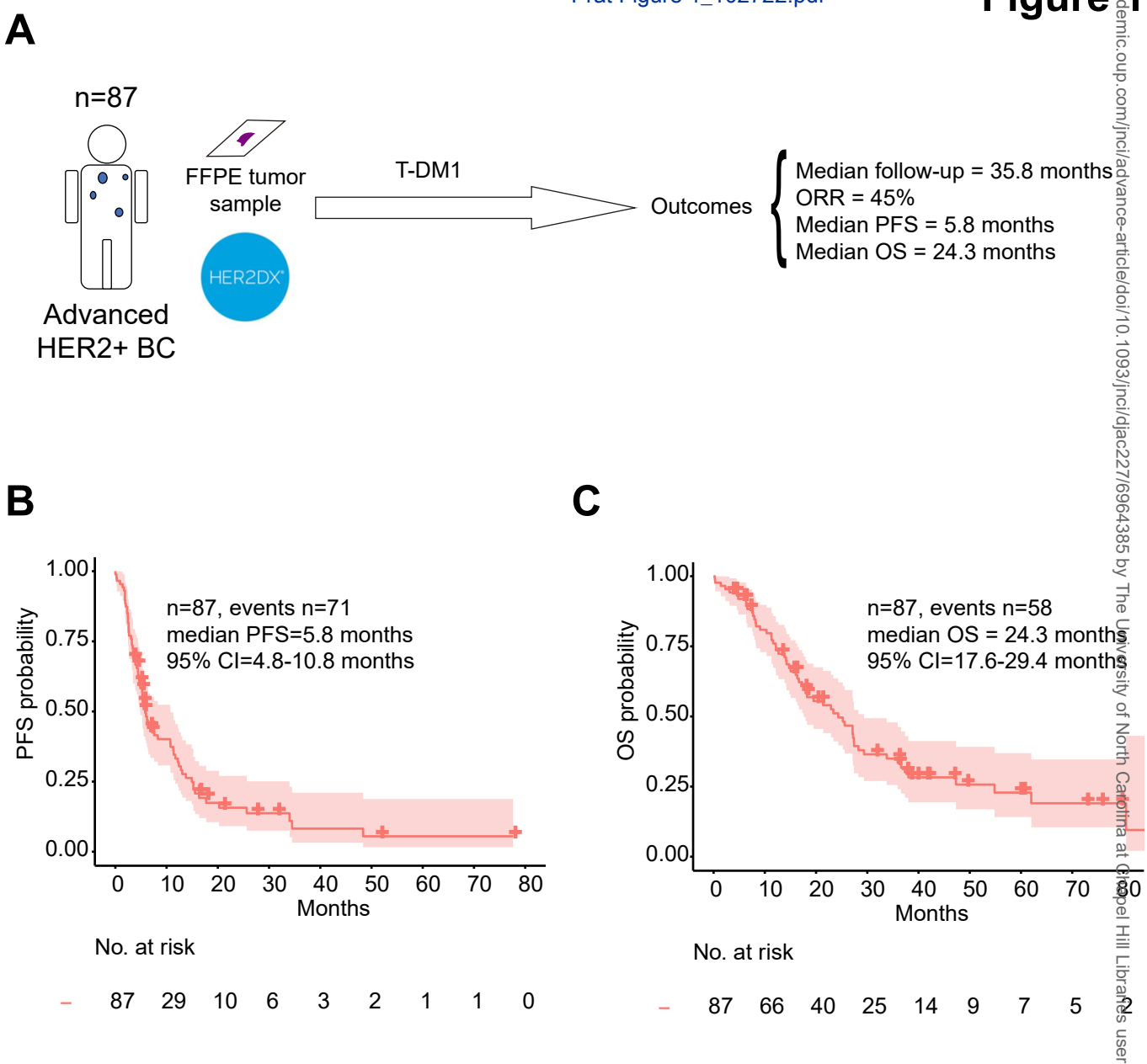
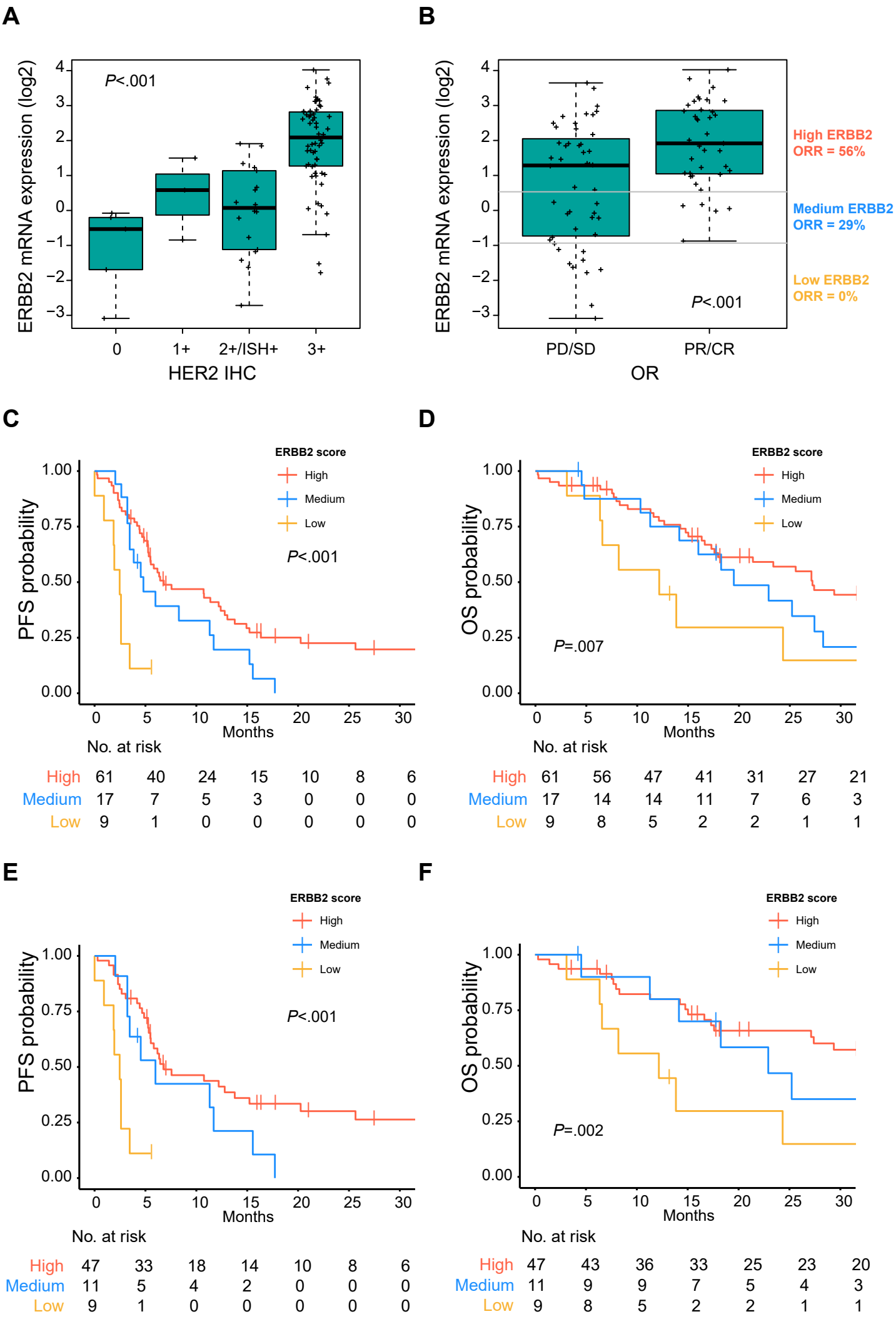


Figure 2



A predictor of response in HER2+ breast cancer – at last!

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The breast cancer world is somewhat fortunate in having biomarkers that are great at selecting patients most likely to respond to targeted therapies. The stratification of breast cancer in to estrogen receptor positive (ER+) and negative disease has stood the test of time. The identification of human epidermal growth factor 2 receptor (HER2) and the development of HER2-directed monoclonal antibodies has resulted in the recognition of another important therapeutic subtype of breast cancer. Both ER and HER2 are good selection markers for therapeutics, however, not all patients with positive status will respond to therapy. The quest for biomarkers predictive of response has led to the realization of the importance of proliferation pathways in ER+ cancers. Most gene signatures for ER+ cancers utilize proliferation related genes, in addition to others, for the stratification of patients to determine the likelihood of recurrence and more importantly the need for chemotherapy. Unfortunately, the same has not been true for tumors overexpressing HER2 and for tumors lacking expression of estrogen (progesterone) and HER2 receptors (triple negative breast cancers).

The challenge of developing gene signatures for HER2+ cancers seemed daunting as a number of mechanisms, in clinical and experimental models, have been shown to be important (**figure 1**) (see Swain et al (1) for a recent review). The first and foremost of these was the definition of HER2 positivity. There is a high but not perfect correlation concordance between IHC assays and between IHC and fluorescent in situ hybridization (FISH) and mRNA levels (2-5). The HER2 pathway is complex with the formation of homo- and hetero-dimers with other family members (1). Dual targeting of HER2 by trastuzumab and pertuzumab results improved response. The HER2-HER3 heterodimer has been implicated in therapeutic resistance. Targeting of the downstream effectors such as

PI3k/AKT and mTOR pathways have been associated with therapeutic benefit. An antibody-drug conjugate against HER3 has been recently developed and shown to be have therapeutic efficacy. However, the benefit did not seem to depend on the levels of HER3 protein. Additional studies are clearly necessary to elucidate the roles of HER2 and its family members in determination of response.

Accumulating evidence has documented a prognostic role of tumor infiltrating lymphocytes (TILs) in HER2+ cancers. TILs are associated with better survival and higher likelihood of obtaining a complete pathological response (pCR) (6-8). A number of gene expression signatures that quantitate immune response have also been described. The expression of checkpoint inhibitors such as PD-L1 has also been assessed in HER2+ tumors and has resulted in several clinical trials studying combination of immune and HER2-directed therapies (1). The importance of immune mechanisms is also highlighted by the role of fragment crystallizable gamma receptor (FcγR)-dependent activities, which include induction of antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), in cancer cell elimination (9). Margetuximab, an FDA approved anti-HER2 Fc-engineered chimeric mAb, targets the same epitope as trastuzumab, but has increased binding to activating FcγRIIIa and decreased binding to inhibitory FcγRIIb (CD32B) (10-12).

In addition to the complexities discussed above, it has should be borne in mind that most anti-HER2 therapeutic trials are in a background of standard of care therapies including surgery and multi-agent chemotherapy. The benefit observed in a (but significant) fraction of patients could be due to one or more of these therapies (i.e. you can cure a patient only once!).

This tangled web of biological and therapeutic factors has presented a formidable barrier to the development of a gene signature to predict risk of recurrence or response to therapy in HER2+ breast cancer. Prat and colleagues seem to have cracked this barrier. They have developed a gene signature using a slightly unusual approach. Instead of using the classical unsupervised approach to identifying key genes associated with adverse outcomes, they have used prior knowledge to develop a “handcrafted” supervised approach. They have recently described a HER2Dx signature to predict the outcomes of patients treated with chemotherapy and trastuzumab(13, 14). The signature incorporates tumor size, nodal status, and 27 genes representing 4 gene expression signatures related to immune infiltration, proliferation, tumor cell differentiation and HER2 amplicon. They developed 2 models, one a prognostic model and second a predictor of likelihood of pathologic complete response (pCR) after anti-HER2-based chemotherapy. The prognostic “risk-score” model is developed using a series of 434 patients and validated in an independent cohort of 268 patients in addition to 5 publicly available datasets. The model performed very well in the validation set for the prediction of disease-free survival ($p=0.002$) with the 5-yr survival in the low-risk group of 97.4%. The pCR model is developed using 116 cases and validated in 2 independent cohort (n=91 and 67 cases). The continuous pCR model, similarly, was significantly associated with pCR ($p<0.0001$). Interestingly, the 2 models, risk-score and pCR-score show only a weak relationship (correlation coefficient =-0.19). in another study Guarneri et al. showed that HER2DX could predict response following neoadjuvant letrozole in combination with dual HER2 blockade with trastuzumab and pertuzumab in early-stage HER2-positive/hormone

receptor-positive breast cancer(14). The limitations of these studies were that they were based on patients with early stage disease treated with trastuzumab based therapies.

In their current work the group, Brasó-Maristany, et al (15), seek to address some of these limitations and document utility of the risk-score patients with advanced disease treated with T-DM1, an antibody drug conjugate. In contrast to the prior work, they find a relatively weak but still significant association (p=0.04) of the risk-score with overall survival in multivariable analyses. The observed result could be due to a combination of several factors including small sample size of the study (n=91). The patient population is also heterogeneous with 46% of the patients having de novo metastatic disease. The remainder of the patients had one or more rounds of prior therapies. The HER2Dx assay was also performed on archival samples and include tissue from primary as well as metastatic sites. Overall, the data has the promise of having utility in a bigger better controlled patient population.

Brasó-Maristany, et al (15) also study the importance of HER2 mRNA, one of the 27 genes in the HER2Dx assay. Low levels of mRNA are found in approximately 10% of HER2+ cases. This is prima facie contrary to the expectation that all clinically HER2+ patients will have high mRNA. However, this is in fact, consistent with prior data. Perez et al (16) studied the correlation of HER2 mRNA performed using 21-gene recurrence score assay and had noted that ~14% of the 901 cases analyzed from the N9831 clinical trial had low mRNA levels. Perez et al also observed only a trend for mRNA levels to correlate with response to trastuzumab (p=0.057). In contrast, in the T-DM1-based EMILIA trial, there was a survival benefit of T-DM1 was greater than compared to control regimen (Capecitabine plus Lapatinib) in patients with high the levels of HER2 mRNA(17).

A similar result was observed in the TH3RESA clinical trial (18). Lastly, in the MARIANNE clinical trial, HER2 mRNA levels greater than median was associated with numerically longer progression free survival (PFS) in all arms of the trial (19). Collectively, these data substantiate the observations made by Brasó-Maristany, et al. (15) who find a strong correlation of the mRNA levels with overall response to T-DM1 (0% in low; 29% in intermediate and 56% in high). An important thing to note is current study was performed on archival primary as well as metastatic samples. Although variability in HER2 protein expression has been documented it is not known what this is for the mRNA. The authors state that based on prior experience (20) they did not expect is to be a major issue.

The main question that arises now is – where does the HER2DX assay fit in to today's practice. The landscape of HER2+ cancers is rapidly evolving with the advent of novel anti-HER2 agents. The results of the DESTINY-04 clinical trial show that trastuzumab deruxtecan is a potent agent even in patients with low levels of HER2 expression (1+ or 2+; FISH negative)(21). A number of other agents such as tyrosine kinase inhibitors (such as FDA approved neratinib, tucatinib) and other novel antibody-drug conjugates are also available(1). It would be interesting to see if HER2Dx could help stratify this increasingly complex landscape and provide patients and their clinicians a tool to personalize and select the most appropriate anti-HER2 agent for therapeutics. The assay could also help sequence these agents to optimize therapeutic response.

In conclusion, the work by Brasó-Maristany, et al. (15) not only builds on the prior data regarding HER2 mRNA levels and likelihood of response but also supports the further development of the HER2DX genomic assay. This likely to become the go-to

assay for assessment of risk of progression in patients with early and advanced stage HER2+ disease, and predicting the likelihood of response to neoadjuvant chemotherapy.

Funding

No funding was used for this editorial.

Notes

Role of the funder: Not applicable.

Author Disclosures: SSB and YGP have no disclosures related to this publication.

Author contributions: SSB: writing – original draft, writing – review & editing. YGP: writing – review & editing

Acknowledgements: Drs. Badve and Gökmen-Polar are supported by Funds from Department of Pathology, Emory University and from Susan G Komen leadership grant (SAC220219) to SSB.

Disclaimers: The thoughts and opinions expressed in this editorial are the author's own and do not reflect those of Oxford University Press or Emory University.

Data Availability

No new data were generated or analyzed for this editorial.

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Figure Legend

Figure 1. Complex biology of HER2-related breast cancers. Response to therapy is multifactorial and impacted by patient variable, HER2 expression and its interaction with other molecular pathways as well as by the therapeutic regimens prescribed for the patient.