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

Fara Brasó-Maristany, PhD[1,*], Gaia Griguolo, MD[2,3,*], Nuria Chic, MD[1,4], Tomás Pascual, MD[4,5], Laia Paré, PhD[6], Julia Maues Degree, N/A[7], Patricia Galván Degree, N/A[1], Maria Vittoria Dieci, MD, PhD[2,3], Federica Miglietta, MD[2,3], Tommaso Giarratano, MD[2,3], Olga Martínez-Sáez, MD, PhD[1,4,8], Mercedes Marín-Aguilera, PhD [6], Francesco Schettini, MD, PhD[1,4], Benedetta Conte, MD[1,4], Laura Angelats, MD[1,4], Maria Vidal MD, PhD[1,4,5,8,9], Barbara Adamo, MD, PhD[1,4,7], Montserrat Muñoz, MD, PhD[1,4,5,8], Esther Sanfeliu, MD, PhD[10], Blanca González, MD, PhD[10], Ana Vivancos, PhD[11], Patricia Villagrasa, PhD[6], Joel S. Parker, PhD[12], Charles M. Perou PhD[13], PierFranco Conte MD, PhD[2,3], Aleix Prat MD, PhD[1,4,5,6,8,9,#] and Valentina Guarneri MD, PhD[2,3,#]

Authors' affiliations

1. Translational Genomics and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain
2. Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, Italy.
3. Division of Oncology 2, Istituto Oncologico Veneto, IRCCS, Padova, Italy.
4. Department of Medical Oncology, Hospital Clinic of Barcelona, Spain
5. SOLTI cooperative group, Barcelona, Spain.
6. Reveal Genomics, Barcelona, Spain
7. GRASP, Baltimore, USA
8. Department of Medicine, University of Barcelona, Barcelona, Spain
9. Institute of Oncology (IOB)-Hospital Quirónsalud, Barcelona, Spain
10. Department of Pathology, Hospital Clinic de Barcelona, Barcelona, Spain
11. Cancer Genomics Group, Vall d´Hebron Institute of Oncology (VHIO), Barcelona, Spain
12. Department of Genetics, University of North Carolina, Chapel Hill
13. Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, USA

*, co-first authors, equal contribution
#, senior authors, equal contribution

#, main corresponding author: Prof. Aleix Prat, Translational Genomic and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Carrer de Villarroel, 170, 08036, Barcelona, Spain. Email: alprat@clinic.cat

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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.

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Notes

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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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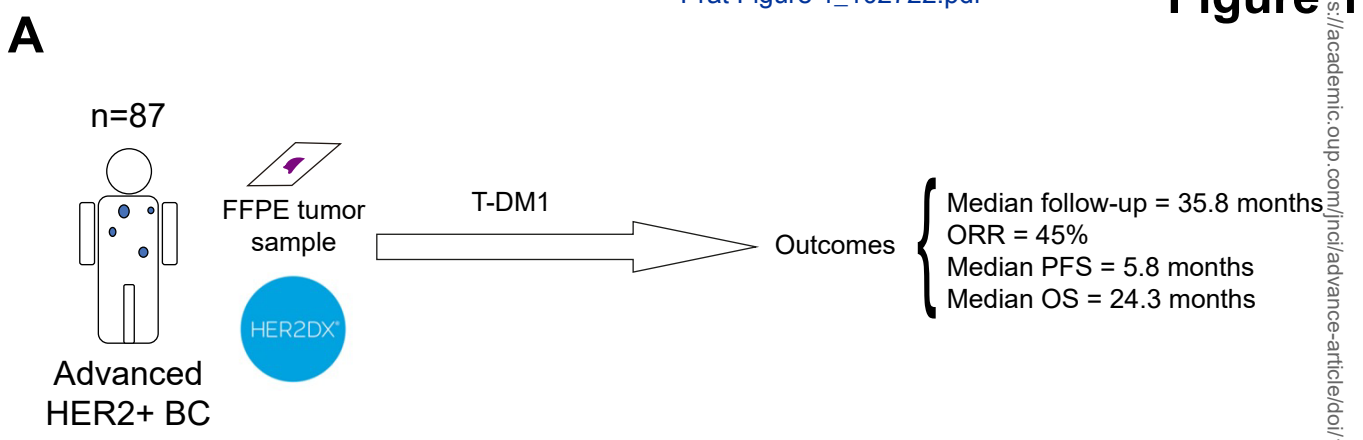
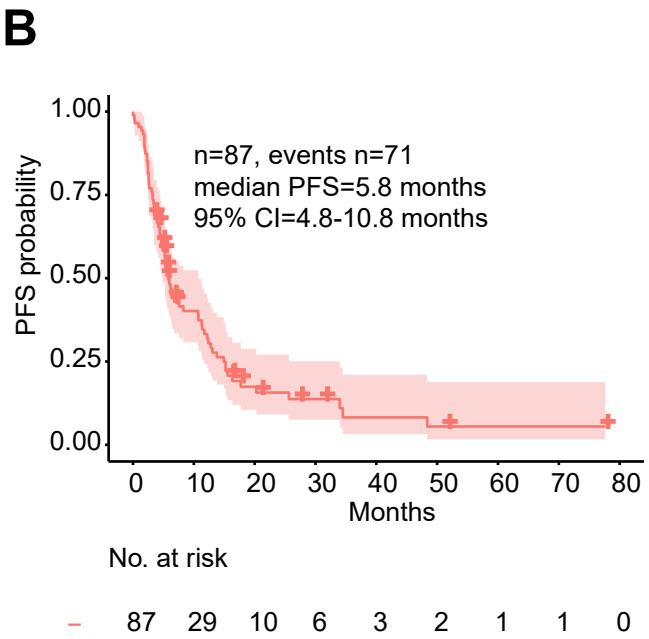
Figure Legends

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





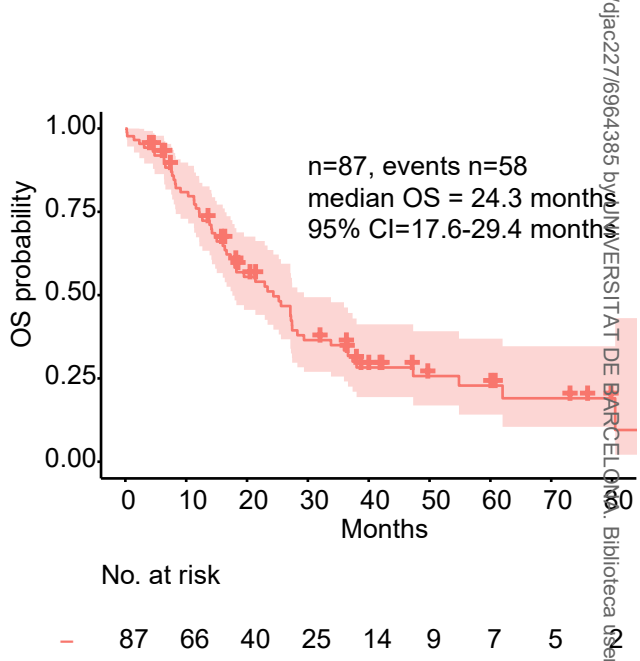


Figure 2