

Relationship between quantitative HER2 protein expression and clinical outcome following trastuzumab treatment in ER-positive and ER-negative sub-groups of patients with metastatic breast cancer

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Abstract 5136

M. Bates¹, J. Sperinde¹, K. Leitzel², S. Ali^{2,3}, E. Fuchs⁴, W. Koestler⁴, C. Singer⁴, W. Huang¹, A. Paquet¹, J. Weidler¹, and A. Lipton²

¹Monogram Biosciences Inc., South San Francisco, CA., ²M.S. Hershey Medical Center, Hershey, PA, ³Lebanon VA Medical Center, Lebanon, PA, ⁴Medical University of Vienna, Vienna, Austria.

Abstract

Background: HER-2/neu over-expression is associated with poor prognosis and with improved outcomes on trastuzumab in patients with metastatic breast cancer (MBC). Estrogen receptor (ER)-mediated signaling in breast cancer cells has been linked to HER2 gene expression, and some ER-positive/HER2- negative patients fail Tamoxifen with HER2-positive disease. These data suggest an interaction between the ER and HER2 signaling mechanisms that may complicate trastuzumab responsiveness in MBC. We have previously reported that quantitative measurements of HER2 protein expression (H2T) using the HERmark assay identifies sub-populations of previously characterized "HER2-positive" patients (by IHC and/or FISH) that have different clinical outcomes on trastuzumab (Leitzel, ASCO 2008; Lipton, SABCS 2008). Here we investigate whether the relationship between quantitative HER2 protein expression and clinical outcomes on trastuzumab are the same in ER-positive and ER-negative sub-populations.

Methods: 102 patients who had received trastuzumab in the metastatic setting and for whom ER status was known were tested by the HERmark assay and H2T levels were determined. Only 62 of 38 ER-positive patients that had received hormonal therapy in the adjuvant setting subsequently received hormonal therapy (exemestane) concomitantly with trastuzumab in the metastatic setting. The HERmark measurements were correlated with TTP and OS using Kaplan-Meier and Cox proportional hazards regression analyses stratified by ER status.

Results: 64 patients in the cohort were ER-negative and 38 were ER-positive. Considering all patients and using an optimized cutoff defined by positional scanning to discriminate high from low H2T, patients with high H2T levels experienced longer TTP than those with lower H2T levels (median TTP 11.1 vs. 4.5 mos., HR=0.43, p=0.0027). Those patients that were ER-negative showed very similar results (median TTP = 11.3 vs. 4.5 mos, HR=0.29, p=0.0023) whereas there was a weaker relationship between H2T and TTP in ER-positive patients (median TTP 11.1 vs. 5.4 mos, HR=0.59, p=0.2). For OS, there was no statistically significant relationship with H2T in the whole population (median OS 35.5 vs. 28.7 mos, HR=0.74, p=0.26) or the ER-positive sub-group (median OS 35.4 vs. 56.6 mos, HR=0.97, p=0.95), but there was in the ER-negative sub-group favoring high H2T (median OS 31.2 vs. 26.6 mos, HR=0.44, p=0.047).

Conclusions: These data suggest that HER2 levels in ER-positive breast tumors may change in response to hormonal treatment and such changes may alter the predictive value of HER2 assessments made prior to treatment. Studies comparing quantitative HER2 levels in ER-positive patients before and after exposure to hormonal therapy are needed. These data are not conclusive given the small number of ER-positive patients studied, and require confirmation in larger datasets.

Total HER-2 expression (H2T) Assay HER-2 homodimer (H2D) Assay

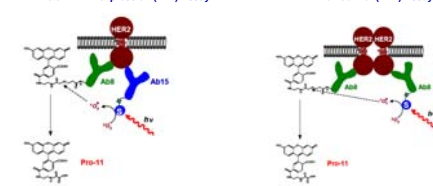


Figure 1: The principle of the HERmark assay – a novel proximity-based technology

A monoclonal antibody specific for a unique epitope of HER-2 is conjugated to a fluorescein VerTag reporter (Pro11) or a photosensitizer molecule (S) by means of a cleavable tether. The photosensitizer liberates singlet O₂ upon irradiation with red light. The free radicals cleave the bonds in close proximity (within approximately 30-100 nm), releasing the VerTag reporter, Pro-11. The Pro11 can then be collected and analyzed on a capillary electrophoretic (CE) array. Each VerTag reporter is designed with a unique charge-mass ratio and can thus be discriminated and quantified by comparison to assay standards. The standard unit of VerTag measurement in tumor samples is relative peak area (RPA) x collection volume (uL) x tumor area (mm²). The HERmark assay has been validated according to CLIA specifications and is run in a CAP-certified clinical reference laboratory.

Characteristics of the RISHQ clinical cohort	
Geographic origin	Vienna, Austria
Centralized HER2 assessment	Yes
Comparator drug collection	Yes
Total number of patients	102
Mean follow-up (months)	34.3 (1.6 - 81.2)
Mean age (range)	55.3 (27.6 - 85.6)
Hormone receptor status	
ER+ / PR+	18 (7%)
ER+ / PR-	18 (7%)
ER- / PR+	21 (9%)
ER- / PR-	23 (9%)
ER unknown / PR unknown	42 (18%)
ER unknown / PR+	2 (1%)
HER2 by IHC	
2+	8 (3%)
3+	66 (30%)
Unknown	2 (1%)
Number of metastatic sites	
≤ 3	62 (56%)
> 3	44 (40%)
Tumor grade	
1-2	2 (2%)
Metastatic sites	
Brain	2 (2%)
Poorly differentiated	54 (52%)
Unknown	2 (2%)
Treatment	
Trastuzumab + chemotherapy	55 (58%)
Trastuzumab only	13 (12%)
Lines of chemotherapy	
First line	78 (74%)
Second line	18 (17%)
Third line	8 (7%)
Unknown	2 (2%)

Table 1. Clinical characteristics of the Vienna cohort.

Distribution of H2T by ER status

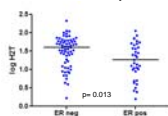


Figure 3. Distribution of H2T by ER status.

Univariate Cox Proportional Hazards for TTP

	log ₁₀ H2T	log ₁₀ H2D
All pts	HR 0.58	0.71
	p value 0.021	0.027
ER neg	HR 0.39	0.52
	p value 0.008	0.007
ER pos	HR 0.71	0.82
	p value 0.38	0.43

Table 2. Univariate Cox for all patients and by ER status using TTP as the outcome measure.

ORR by H2T quartiles

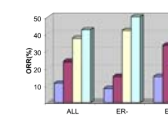


Figure 4. Objective response rate by ER status. ORR = CR+PR/CR+PR+SD+PD.

ORR by H2D quartiles

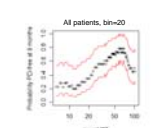


Figure 5. Kaplan-Meier analyses of TTP and OS for H2T using the optimized H2T cutoff in all patients, and by ER status. High HER2 refers to H2T levels above the optimized cutoff.

Figure 6. STEPP analyses for all patients and by ER status. STEPP= sub-population treatment effect pattern plots.

H2T cutoff

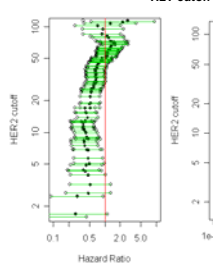


Figure 2: H2T and H2D cutoff identification by positional scanning and selection of the breakpoint associated with the lowest p-value for TTP. For H2T, the optimal cutoff is 13.8. For H2D, it is 2.95.

H2D cutoff

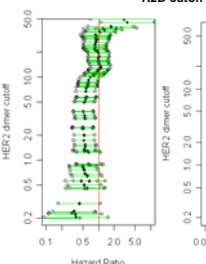


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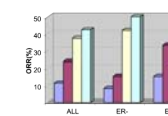


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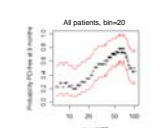


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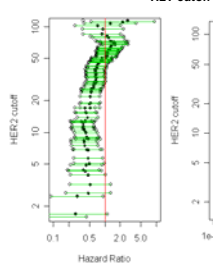
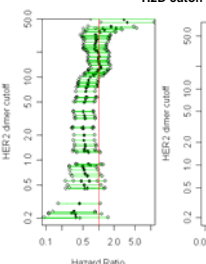


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H2D cutoff



Results summary

In this cohort of trastuzumab-treated patients with HER2-positive MBC, those patients with ER-negative disease have higher levels of HER2 than patients with ER-positive disease, although the distributions overlap significantly.

Quantitative measures of H2T and H2D correlate with objective response (ORR) to trastuzumab in ER-negative patients but not in ER-positives.

Kaplan-Meier analyses demonstrate a significant correlation between quantitative H2T and TTP as well as OS in ER-negative patients but not in ER-positives.

STEPP analyses show a better correlation between H2T and outcome at 9 months in ER-negative patients than ER-positives.

Cox Proportional Hazards analyses demonstrate a significant relationship between H2T or H2D and TTP in ER-negatives, but not ER-positives.

Conclusions

These data are consistent with the notion of linkage between the ER and HER2 signaling pathways. Overall, patients with higher H2T levels experienced better outcomes on trastuzumab. This was especially true for the ER-negative sub-group.

The lack of correlation between HER2 measurements made prior to the initiation of hormonal therapy for ER-positive disease may result from up-regulation of HER2 signaling as an adaptive mechanism in the face of ER pathway antagonism. This possibility is of particular interest in view of the fact that approximately 85% of ER-positive patients did not receive trastuzumab and hormonal therapy concurrently in the metastatic setting.

However, these data could also be the result of statistical artifact relating to the small sample size of the cohort, particularly affecting the ER-positive sub-group (N=38).

Additional studies employing quantitative measures of HER2 expression and dimerization in larger populations of patients are warranted to explore the relationship between ER and HER2 signaling under selective pressure from anti-estrogen therapies. In particular, re-sampling at the time of therapeutic failure may offer an opportunity to witness the adaptive changes that occur in breast tumors under selective pressure from drugs.

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