Commentary

Estrogen receptor and human epidermal growth factor receptor-2 quantification and efficacy to trastuzumab

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Trastuzumab in breast cancer exemplifies the paradigm of successful targeted therapy. This anti-HER2 monoclonal antibody reduces the risk of recurrence by half when combined with standard chemotherapy (1-3). Its target—HER2—is overexpressed in 20% of breast cancers and testing for HER2 positivity is the sine qua non for anti-HER2 therapies. Consensus guidelines have been developed for the definition of HER2-positive (HER2+) tumors. HER2 protein expression has to be 3+ by immunohistochemistry (IHC) or the gene has to be amplified by fluorescence (FISH) or chromogenic (CISH) in situ hybridization (4).

These benchmark criteria are still considered current despite the fact that not all HER2+ patients derive similar benefit from trastuzumab-based therapy. The identification of a robust clinical or molecular predictor of benefit from trastuzumab has proven challenging. A variety of mechanisms of resistance to this therapy have been described, but none are currently used for guiding clinical decision-making (5). Even HER2, the most obvious biomarker, was highly ambiguous up until it was evidenced that even HER2-negative tumors (centrally determined) may derive benefit from trastuzumab (6).

The article "Effects of Estrogen Receptor and Human Epidermal Growth Factor Receptor-2 Levels on the Efficacy of Trastuzumab: A Secondary Analysis of HERA Trial," by Loi *et al.* (7) is one of the most recent ambitious attempts aimed at identifying subgroups of patients with varying sensitivity to anti-HER2 therapy. Starting from a priori hypothesis that benefit from trastuzumab is dependent on the level of ER expression, the researchers study objective was to determine the magnitude of trastuzumab benefit according to the quantitative levels of ER and HER2 using samples collected from a subset of Herceptin Adjuvant (HERA) patients.

The HERA trial was an international, intergroup, open-label, phase 3 randomized trial comparing treatment with trastuzumab for either 1 year or 2 years versus observation alone (i.e., without trastuzumab) after standard neoadjuvant chemotherapy, adjuvant chemotherapy, or both in 5,102 patients with HER2+ early-stage breast cancer (8). Loi *et al.* divided the original study population in two cohorts (based on specimen availability) where they respectively addressed the impact of ER expression and HER2 amplification (cohort 1: 3,037 patients) and ESR1 and HER2 transcript levels (cohort 2: 615 patients) on disease-free survival (DFS) and overall survival (OS). ER protein content was defined as either positive (>10%) or negative (<10%) whereas the FISH ratio and copy number groups were defined as FISH ratio low (≥2 to <5), FISH ratio high (≥5), HER2 copy number low (≥4 to <10) and HER2 copy number high (≥10).

The DFS hazard ratios (favoring trastuzumab) were similar across the different subgroups using multiple statistical analysis methodologies, including the intent-to-treat (ITT) analysis and the statistical analyses used to adjust for the significant patient crossover to trastuzumab that occurred in the HERA study (censored and inverse probability weighted or IPW). However, an OS hazard ratio of 1.27 (favoring observation) was observed in the ITT analysis (but not in the IPW and censored analyses) in the ER+/FISH low subgroup. The interaction between treatment and the combination of ER IHC and HER2

copy number was not significant, possibly further stressing the importance of polisomy. Analyses in cohort 2 mirrored the results of cohort 1, by showing that tumors with the highest ESR1 expression (3rd tertile) had a lower benefit to trastuzumab.

The results of this study—although not practice changing—confirm the close relationship between ER and HER2 pathways. Molecular subgrouping of breast cancer had already previously evidenced that not all HER2+ tumors belong to the "HER2-enriched" subtype (9) (predominantly ER negative, greater responders to HER2 inhibition). The subgroup ER+/FISH low identified by the researchers may overlap—at least in part—to HER2+ breast cancer with a LUMINAL B subtype or the group defined in the study by Pogue-Geile (10) as high ESR1/intermediate ERBB2 transcript levels which also showed limited benefit to trastuzumab.

Due to the inverse relationship between ER and HER2, one could speculate that this subgroup of HER2+ tumors with poor response to trastuzumab characterized by high ER expression and low gene amplification by FISH just has less target protein despite being classified as HER2+ by standard testing. IHC is a qualitative technique and the inter-laboratory and inter-observer variability are well described (11,12). With FISH, we might well achieve some level of quantification by directly counting gene copies in the nuclei but, (I) we currently use it as a qualitative tool (<2 *vs.* >2) and (II) the levels and pattern of amplification may directly affect the amount of protein expressed.

These considerations raise one important question: is the current approach adopted to classify a tumor into HER2 positive or negative actually accurate?

In contrast with classical targeted therapies where the target is a protein with activating mutations (BRAF in melanoma, EGFR in lung cancer), the fundamental principle of HER2 targeted therapy in breast cancer is to specifically damage tumor cells that express the target protein in much greater amounts in comparison to normal cells. Surely then, sensitivity to trastuzumab must relate to HER2 levels of expression. Current HER2 testing approaches (IHC and FISH) are however far from quantitative. There is an increasing body of evidence indicating that the levels of HER2 in HER2+ tumors can influence the response to HER2-targeted therapy (13-16), converging to the common conclusion that 'more HER2, more response'. If one possible criticism could be made surrounding the Loi *et al*. elegant study, it might be that ER and HER2 assessments were not really "quantitative" despite the claims of the authors. The crosstalk between ER and HER2 is not an "on/off" type but probably depends on variations of the levels of expression of the two proteins. In the context of ER-positivity, ER expression levels may directly affect the level of suppression of HER2. It would have been tremendously impacting had the HER2 protein in the HERA trial been quantitated by available platforms such as mass spectrometry or Veratag assays.

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Footnote

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