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Letter to the editor regarding 'Seol H, Lee HJ, Choi Y, *et al*. Intratumoural heterogeneity of *HER2* gene amplification in breast cancer: its clinicopathological significance'

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To the Editor: In Seol *et al*,[1] the authors provide a clinicopathologic analysis showing that intratumoral heterogeneity of *HER2* gene amplification is associated with short disease-free survival. They conclude that it is likely that intratumoral heterogeneity is a surrogate for chromosomal instability, and thus a poor prognosis. This result would appear directly to conflict with the study of Bartlett *et al*,[2] showing that patients with tumors that are uniformly *HER2*-amplified do worse than those with heterogeneity (eg, 30–50% of cells with a ratio >2.2). Seol *et al*[1] attribute this difference to a variation in study design—that they have selected their heterogeneous cases from tumors that were already classified as *HER2*-amplified on whole-tissue sections. To this reader, an alternative interpretation presents itself, which takes into account patient treatment, as well as one study[3] not cited by Seol *et al* (See Table 1).

Table 1 Prognostic significance of '*HER2* heterogeneity' accounting for treatment

Study	*Prognostic significance of 'Heterogeneous' or 'Borderline' HER2-amplification*	*Non-amplified cases Included*	*Treated with neoadjuvant/ adjuvant chemotherapy*	*Treated with trastuzumab*
Seol *et al*[1]	Poor[a]	No	93%	26%
Bartlett *et al*[2]	Favorable[b]	Yes	0%	0%
Dowsett *et al*[3]	No Difference[c]	No	100%	100%[d]

[a]Compared to uniformly *HER2*-amplified.
[b]Compared to uniformly *HER2*-amplified; intermediate between amplified and non-amplified.
[c]Borderline/low-*HER2*-amplified compared to highly *HER2*-amplified.
[d]Comparison based on single arm of study.

From Table 1, it appears that intratumoral heterogeneity, in and of itself, is not a poor prognostic marker at all.[2] Rather, high/unequivocal *HER2* amplification is a favorable predictor of response to (antracycline-based) chemotherapy—a result that has been well documented.[3,4] Moreover, patients with low-*HER2*-amplification—and heterogeneity, perhaps—still benefit from trastuzumab in addition to chemotherapy.[1,3]

Seol *et al*[1] rightly highlight the importance of determining the *HER2* amplification status accurately, both overall and taking into account intratumoral heterogeneity. Based on our own work, a fully satisfactory definition of heterogeneity has not been forthcoming. A persistent problem is how to distinguish bonafide heterogeneity from statistical artifact.[5] Both Bartlett *et al*[2] and Seol *et al*[1] raise the possibility of examining 'regional heterogeneity'. The current guidelines address this by recommending that distinct (clustered)

subregions with differences in *HER2* status be scored separately.[6] Further work is needed to define the most revealing testing parameters with respect to prognosis, trastuzumab response, and chemotherapy response.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Reply to 'Intratumoral heterogeneity of HER2 gene amplification in breast cancer: its clinicopathological significance'

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To the editor: We read with great interest the comments by Arena *et al.* and Chang in reference to our paper, 'Intratumoral heterogeneity of *HER2* gene amplification in breast cancer: its clinicopathological significance'.[1] Their letters focused on different issues of the *HER2* intratumoral heterogeneity in breast cancer.

Arena *et al.* questioned about the best way to write *HER2* reports for the clinician and suggested that *HER2* analytical report should be completed with a critical evaluation of the results about *HER2* genetic heterogeneity. Although the clinical relevance of *HER2* genetic heterogeneity is not established in breast cancer, we agree that *HER2 in situ* hybridization report should include not only overall average ratio of *HER2*/CEP17 and average *HER2* gene copy number, but also information about *HER2* genetic heterogeneity. However, there are some issues to be addressed in the definition of *HER2* genetic heterogeneity proposed by 2009 College of American Pathologists expert panel, which indicates the presence of tumor cells with *HER2*/CEP17 signal ratios >2.2 (or >6 *HER2* signals per cell when using a probe for *HER2* only) in 5–50% of the tumor cells tested.[2] If 20 cells are counted and 1 tumor cell is identified with a *HER2*/CEP17 >2.2, the tumor is diagnosed to have *HER2* genetic heterogeneity. However, a recent study revealed that the tumor cells with 3:1 *HER2*/CEP17 ratio, which may reflect technical issues, were determining factor for heterogeneity in 46% of heterogeneous cases.[3] Furthermore, Allison *et al.*[4] reported that the ratio criteria and the criteria based on *HER2* signals per cell for definition of *HER2* genetic heterogeneity were not equivalent and the ratio-based definition resulted in large numbers of non-amplified cases being classified as heterogeneous. Thus, to avoid artifactual heterogeneity caused by technical issues, such as nuclear truncation and inadequate hybridization, cutoff values of percentage and cell ratio for *HER2* genetic heterogeneity need to be validated. Furthermore, the number of cells to be counted and the fields to be selected for counting should be clearly defined through robust evidence.

HER2 intratumoral heterogeneity appears as two forms; distinct clusters of amplified cells and admixture of amplified and non-amplified cells. Distinct *HER2* amplified clones in a non-amplified tumor, which was defined as *HER2* regional heterogeneity in our study, should be scored separately, as proposed previously.[2,5] *HER2* regional heterogeneity can be assessed by scanning the entire tumor section before selection of fields to be counted and matching with HER2 immunohistochemistry (IHC). If the tumor has differentially amplified or stained area, the regions should be included in the counting. From this point of view, silver *in situ* hybridization has an advantage to evaluate *HER2* regional