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HER-2 amplification is highly homogenous in gastric cancer—reply

To the Editor,

We very much appreciate the comments by Bilous et al further expanding the important discussion on HER2 amplification and overexpression in gastric cancer. The data provided by Bilous et al are to a large extent consistant with our findings. For example, their unpublished tissue multi array (TMA) study of 170 gastric cancer cases showed strikingly similar results: In 19.4% (n = 33) of the cases gene amplification strongly correlated with protein overexpression (as compared to 16% in our series of 166 gastric cancers). Bilous et al also strongly corroborate our observation of a strong association of protein overexpression and gene amplification; 23 of their 24 3+ cases (95.8%) and 4 of 9 2+ cases (44%) showed gene amplification. Our study revealed 100% amplification in 22 immunohistochemistry (IHC) 3+ and 83.3% (5/6) IHC 2+ cases [1].

In their letter, Bilous et al express concern on heterogeneity representing a significant problem in HER2-positive gastric cancer. Specifically, they report about 30% immunohistochemical heterogeneity in HER2-positive tumors in a prospective phase III study on more than 3000 patients. We obviously agree with Bilous et al that heterogeneity of HER2 amplification and overexpression can occur in stomach cancer. This is not in conflict with our data provided in the article by Marx et al [1], as our series of highly homogeneously amplified cases consisted of only eight cases. We even agree that our tissue microarray approach to identify HER2-positive cases may have led to an accumulation of highly homogeneous cases as these are most likely found by the TMA technique. However, also based on our diagnostic experience in the context of clinical trials, we remain very certain that HER2 amplification in gastric cancer represents an exceptionally homogeneous phenomenon.

We recently summarized methodological IHC problems for HER2 extensively (Sauter et al [2]). We view it very possible that pre-processing issues are responsible for much of the observed immunohistochemical heterogeneity of HER2 in gastric cancer. Even in specialized centers, fixation of gastric cancers is difficult to standardize because formalin penetration varies drastically between small biopsies and large resection specimens. Preprocessing variability further increases in case of multicentric trials such as the population described by Bilous et al (ASCO 2009 Abstract xx).

Given the importance of the subject, we would eventually like to specifically respond to the five final statements made by Bilous et al in their letter:

Gastric cancers are more heterogeneous in the level of HER2 protein expression and/or gene amplification than breast cancers.

Given the low frequency of heterogeneity of HER2 in breast cancer (about 5%), it is well possible that this statement is true. However, it is still remarkable that HER2 amplification and overexpression is homogenous in most gastric cancers. HER2 amplification is much less homogenous in other tumors like bladder cancer [3] and colorectal cancer (unpublished own data). Other amplification targets like EGFR in lung cancer are only rarely homogenous (unpublished own data).

A specific IHC scoring system has been proposed for use in gastric cancers modifying the system used in breast cancer [3].

It is important to note, however, that this scoring system has not been clinically validated to be superior as compared to other scoring systems or fluorescence in situ hybridization results.

The correlation between IHC and fluorescence in situ hybridization results is less consistent than seen in breast cancer. A significant number of gastric cancers with HER2 protein at the 0/1+ level show gene amplification.

We do not agree that the available data allow such a conclusion. There are many possible causes that can lead to artificially reduced immunogenicity. Studies are needed that compare expression of HER2 in highly characterized, well-preserved, unfixed tissues to thoroughly compare HER2 RNA, protein, and DNA levels, as it has been done in breast cancer. Convincing data on differences in the regulation of HER2 expression between gastric and breast cancer are so far missing.

It is difficult to draw meaningful conclusions from small TMA cores when the biomarker is so heterogeneously distributed and the TMAs do not accurately reflect the distribution of HER2 in large population studies. Thus, only 2.4% of the cores in the study by Marx et al were assessed as IHC1+, whereas in the ToGA[1] trial, the distribution of IHC1+ in more than 3000 cases of gastric cancer was 18.6%.

We very much disagree with the notion that meaningful conclusions from TMA studies cannot be made [4]. First of all, it is quite uncertain whether a heterogeneous 1+ HER2 positivity, which is discovered on large sections but not on TMAs, reflects true overexpression and has clinical relevance. The only large-scale study comparing findings on large sections and TMAs with clinical data found that p53 positivity that was only seen on large sections but not on TMAs was completely unrelated to breast cancer prognosis while separate analyses from 4 different TMAs from the same cohort found a marked association between p53 IHC and clinical outcome [5]. Moreover, we believe that TMAs composed of cores from several different tissue blocks of one cancer may be much more suited for molecular analysis of potentially heterogeneous targets than traditional large sections. We proposed such an approach in 2002 and 2003 [6,7].

Fixation artifact is unlikely to be the main factor accounting for staining variability in the tumors because biopsies were used as opposed to resection specimens and fixation was likely to have been better controlled.

Fixation artifacts are always a very likely an explanation for unexpected IHC results. They even can occur in small biopsies, for example, because some centers prefer fast fixation procedures to satisfy their customers. It is also our experience that variable fixation habits between countries represent a major issue for molecular pathologists in case of international multicenter studies. For example, in a world-wide breast cancer trial, we confirmed 3+ IHC HER2 positivity in less than 80% of the cases [8].

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