Reviewer's report

Title: SISH/CISH or qPCR as alternative techniques to FISH for determination of HER2 amplification status on breast tumors core needle biopsies: A multicenter experience based on 840 cases

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Reviewer: Carsten Denkert

Reviewer's report:

The manuscript by Jacquemier et al. offers data on SISH, CISH, and qPCR analysis as alternative techniques to the gold standard FISH for the determination of HER2 amplification status in a multicenter study on a large number (n=840) of paraffin-embedded core biopsies of breast tumors. The presented data shows a high concordance between all alternative techniques tested with FISH analyses. This is of special interest for the newly designed qPCR method, as the authors state that it is reliable, easy to perform and less expensive than ISH tests.

Minor Essential Revisions:

Abstract:

In the methods section of the Abstract, the number of centers in which the alternative techniques SISH and CISH were performed are given in parentheses, but the number of centers that performed qPCR is not mentioned.

Background:

Reference 1 is missing in the text. (The first paper referred to in the introduction is the article referred to as number 2 in the reference list).

Methods:

1. Please give a list of antibodies used in the study.

2. As the qPCR analyses are of special interest in your study, it would be advantageous to give more details on the method used, for example the sequences of the primers. It would also be interesting to learn how the data was interpreted. How did you calculate cutoff-points? Were the cutoff-points pre-defined? Since the participating institutes used their own qPCR platform, did
you have to define different cutoff-points for the different instruments?

3. Which statistical tests were used for the calculation of sample size, concordance and CI?

Results:

1. Figure 2 of the ASCO/CAP guidelines stated that patients with HER2/CEP17 FISH amplification ratio \# 2.0 were eligible for the trastuzumab adjuvant trials. Therefore it would be interesting to perform additional statistical analyses using a ratio \# 2.0 as a cutoff-point.

2. In the paragraphs on predictive value, please refer to the respective table the data is given in.

3. Please include graphics on IHC and ISH staining, as well as PCR data.

Tables:

1. Recheck the formatting of Table 1 and Table 2.

2. In the legend of Table 2 it should say: “double and mono probe FISH.”

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.