**Author’s response to reviews**

**Title:** Plasma HER2ECD a promising test for patient prognosis and prediction of response in HER2 positive breast cancer: Results of a randomized study - SAKK 22/99

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**Author’s response to reviews:**

Dear Dr. Solera,

Please find enclosed our revised manuscript and here below point-by-point the answers to the reviewers’ comments. We are thankful for all the constructive comments that lead to an improved version of our manuscript.

The section “Funding” has been completed as shown here below. Thank you for this advice.

**Funding**

This work was supported by SAKK (Swiss Group for Clinical Cancer Research), that reimbursed in part the costs of the kits, the cost of sample collection at the centers and the salary of those performing statistical analyses and manuscript revising. The former Stiftung Tumorbank Basel (STB foundation) and afterword the Institute for medical genetics and pathology at the University Hospital of Basel supplied the infrastructure and personnel for logistics, test performance and sample storage. Study design and manuscript writing were scientific efforts of the researchers involved.
Answers to Reviewer 1
We thank Dr. Elda Tagliabue for her positive review and for her supportive suggestions.

1.1. Although SAKK22/99 clinical study has been already published, it would be better to detail in M&M the treatment of patients enrolled in Arm A and B.

We added the following sentence explaining the treatment modalities:
The primary endpoint of this superiority trial was time to progression (TTP) on combined trastuzumab-chemotherapy (TTP-TChemo) in both arms. Secondary endpoints included response rate, TTP, overall survival, quality of life and toxicity. The T loading dose of 4 mg/kg iv was followed by 2 mg/kg iv weekly. In the initial 1st-line population (n=84), chemotherapy was weekly paclitaxel 90 mg/m2 iv (3/4 weeks). After the amendment, chemotherapy was at the investigator’s choice (taxanes, vinorelbine, cisplatin) according to label indications/schedules. Chemotherapy could be stopped after ≥6 cycles in responding patients or after unacceptable toxicity, trastuzumab was continued until progression.

1.2. The number of cases in which correlation between HER2ECD values before and after first Trastuzumab treatment was carried out should be indicated.

The number of cases has been added in the first results’ paragraph:
The HER2ECD levels just before (n=131) and after (n=113) trastuzumab infusion varied within the normal repeated measurements variance independently from their levels. The detected ranges varied from 5-1427ng/ml (median 26ng/ml; mean 81ng/ml) and 5-1543ng/ml (median 27ng/ml; mean 76ng/ml) in samples before and after the first trastuzumab infusion with a resulting Pearson correlation coefficient of 0.995. This extremely high correlation is independent from the treatment arm (Arm A: r=0.997; Arm B: r=0.993). Subsequently, we considered the mean of these two detected values as baseline if both values (n=113) were available.

1.3. Since levels of HER2ECD only depend on tumor mass, did the authors investigate plasma values according to the number of metastatic sites?

Thank you for this important suggestion. We investigated this aspect, but the results were negative. The missing correlation between the number of metastatic sites and the HER2ECD values (p=0.371) is now added in the manuscript at the end of the first paragraph of the result section.

No significant difference was found between the HER2ECD baseline values and other available parameters such as ER and PR expression (P=0.64 and P=0.68), bone disease (P=0.40) or number of metastatic sites (P=0.37). Only a positive trend was observed between HER2ECD levels and the presence of visceral disease (P=0.05).

Answers to Reviewer 3
We are grateful to Dr. William Jacot for all the suggestions he made to optimize the manuscript.

3.1. In the abstract, the abbreviation TTP-TChemo has not been explained previously and need to be modified
The abbreviation has been appropriately explained by adding “time to progression on combined trastuzumab-chemotherapy.”

3.2. In the background, P4L8-10, HER2 is the target of MoAbs, but of TKIs too. I suggest to modify the sentence accordingly:

Thank you, for this observation. Indeed, this relevant information was missing and it is now added.

HER2 represents the target of monoclonal antibodies, such as trastuzumab, pertuzumab and T-DM1, effective in the cure of patients with HER2 positive BC [5, 6] as well as of tyrosine kinase inhibitors (TKIs) [7, 8].

3.3. Under methods P4L54, the PD abbreviation has not been justified.

The abbreviation is now explained.
In short, women with histologically proven HER2-positive advanced BC without previous trastuzumab treatment or brain/meningeal involvement or concomitant serious diseases were randomly assigned (1:1) to trastuzumab alone followed, at progressive disease (PD), by combination with chemotherapy (Arm A) versus the upfront TChemo (Arm B).

3.4. Regarding the results section, a table resuming the main clinicopathological data and the correlations would be useful. In addition, was there a correlation between the number of metastatic sites (as a correlate of tumor burden) and HER2ECD values?

We now added Table 1 summarizing the main clinicopathological data. Since in general the HER2ECD did correlate only with the HER2 expression levels of the primary tumour (significantly more plasma levels when the values of the primary tumors was Dako 3+ as compared to the Dako 2+) and with the presence of visceral disease, we did not add a table with correlation. We hope Dr. Jacot can agree with that. Regarding the correlation with the number of metastatic sites, we couldn’t find any significant correlation (p=0.37) and added this observation at the end of the first paragraph in the result section. (See also answer to question 3 of Reviewer 1)

3.5 In the monitoring HER2ECD section, in the results section, is it possible to evaluate the anticipation time for HER2ECD rising before disease progression? It would make this marker far more valuable. If not available, would be a good point of discussion for the perspectives.

We are grateful for this observation and suggestion. As described under inclusion criteria according to our protocol plasma sampling occurred at baseline, after 3 weeks, at first response evaluation (8-9 weeks) and at tumor progression. Figure 3 shows that HER2ECD levels of patients in Arm A, in general remained almost constant till the first assessment performed at 8-9 weeks (circa 2 months). In half of the cases progression occurred after 6 or more months. It is impossible to know how long before the HER2ECD levels started to raise. Subsequently as suggested, we added in the discussion the following sentence:

According to protocol plasma collection timelines (baseline, after 3 weeks, at first response evaluation (8-9 weeks) and at PD) it is not possible to know how long before PD the HER2ECD
levels started to raise. Prospective studies should integrate monthly detections in order to estimate the possible anticipation of chemotherapy administration.

We hope this improved version will be now suitable for publication.

If this is the case, we kindly ask you to add the annotation that the two last author share the last authorship of this manuscript. Unfortunately, I couldn’t find the way to do so during the electronic submission.

Looking forward to hearing a positive final decision

Yours sincerely,

Serenella Eppenberger-Castori