Author's response to reviews

Title: Differential survival following trastuzumab treatment based on quantitative HER2 expression and HER2 homodimers in a clinic-based cohort of patients with metastatic breast cancer.

Authors:

Masakazu Toi (toi@kuhp.kyoto-u.ac.jp)
Jeff Sperinde (jsperinde@monogrambio.com)
Weidong Huang (whuang2@monogrambio.com)
Shigehira Saji (ss-saji@wa2.so-net.ne.jp)
John Winslow (jwinslow@monogrambio.com)
Xueguang Jin (xjin@monogrambio.com)
Yuping Tan (ytan@monogrambio.com)
Shinji Ohno (sohno@nk-cc.go.jp)
Seigo Nakamura (seigonak@luke.or.jp)
Hiroji Iwata (hiwata@aichi-cc.jp)
Norikazu Masuda (nmasuda@alpha.ocn.ne.jp)
Kenjiro Aogi (kaogi@shikoku-cc.go.jp)
Satoshi Morita (smorita@urahp.yokohama-cu.ac.jp)
Christos Petropoulos (cpetropoulos@monogrambio.com)
Michael Bates (mbates@monogrambio.com)

Version: 2 Date: 20 August 2009

Author's response to reviews: see over
We thank the referees for their very constructive and thoughtful reviews of the manuscript. We found the reviews extremely helpful and have revised the manuscript accordingly. Our point-by-point responses to the reviewers’ comments are given below.

**Points raised by Reiki Nishimura**

1.

2.

3. **Subgroup analyses of patient characteristics by H2T.**

As suggested, we have conducted subgroup analyses of patient characteristics by H2T (<median / >median value of HER2-expressing), which have been summarized in Table 1. In addition, we have analyzed the relationship between H2T and HER2 score on repeat testing and have described it in the Results section.

4. **The relationship between OS and IHC score on repeat testing.**

We did not find any significant differences in OS between IHC subgroups (log-rank test: p = 0.610).

5. **The results of univariate analysis for OS.**

As suggested, we have added a new Table 2 to show the results of univariate analyses for OS and have added statements about them in the Results section.

6.
**Points raised by Shaheenah Dawood**

**Minor Essential Revisions**

1. 

2. 

3. 

4. **P values, hazard ratios, and confidence intervals.**

   As suggested, we have clarified the Results section by adding p values, hazard ratios, and confidence intervals.

5. **P value and number at risk in Figure 2.**

   As suggested, we have added the p value and the number at risk in Figure 2.

6. **The number of lines of chemotherapy in Table 1.**

   As suggested, we have added the number of lines of chemotherapy before trastuzumab was initiated in Table 1.

7. **Median durations of trastuzumab treatment and patient follow-up.**

   As suggested, we have added the median durations of trastuzumab treatment and patient follow-up in the section of Description of the clinical cohort.

8. **Median survival time and two year survival rate of the cohort.**

   As suggested, we have added the estimates of two year survival rates as the whole cohort and by H2T subgroup in the Results section and Figure 2. Because our data did not reach median survival time as the whole cohort, we did not report the estimate for it.

9. **The number of patients with recurrent or advanced disease.**
As suggested, we have added a distribution of the patients with recurrent or advanced disease in Table 1.

10. Relationships between brain metastases with trastuzumab treatment and HER2 expression level.

According to the study design protocol, patients who were clinically confirmed to have any symptom due to brain metastases at the initiation of trastuzumab treatment were not enrolled onto the study. No significant correlation was found between brain metastases and HER2 expression level ($p = 0.611$).

11. The number of specimens that had HER2 2+ or 3+.

As suggested, we have added the number of specimens of HER2 test in Table 1.

Major Compulsory Revisions

1.

2. The analyses repeated excluding patients with HER2 0 or 1+.

As suggested, we have carried out the analyses to examine the relationship between the HER2 expression level and OS in patients with HER2 2+ or 3+. We could not obtain any statistically reliable result, because of small number of patients in the subgroup. Therefore, we have discussed in the Discussion section on this issue.

3. Cox analysis using a covariate defined as hormone receptor positive or negative.

As suggested, we re-conducted the Cox analyses replacing estrogen and progesterone receptor variables with their composite variable, hormone receptor positive vs. negative. We have revised old Tables 2 and 3 and have summarized the results in new Tables 3 and 4.

4a.

4b. The p-values for the associations between OS and H2D and H2D/H2T.
As suggested, we have added the p-values in the Results section.

4c. The number of patients in the higher and lower HER2 expression subgroups.

As suggested, we have added the numbers of patients in the HER2 expression subgroups in the Results section and new Table 4.

5.

6.

7. The influence of chemotherapy on survival.

As we showed in new Table 2, the absence of chemotherapy did not affect overall survival ($p = 0.693$). We have added a statement on this point in the Results section. There was no remarkable result on the relationship with responses of chemotherapy.