Author’s response to reviews

Title: Inter-observer reproducibility of HER2 immunohistochemical assessment and concordance with fluorescent in situ hybridization (FISH): pathologist assessment compared to quantitative image analysis

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Author’s response to reviews: see over
Cover Letter

April 28th, 2009

RE: “Inter-observer reproducibility of HER2 immunohistochemical assessment and concordance with fluorescent in situ hybridization (FISH): pathologist assessment compared to quantitative image analysis” by Gulisa Turashvili, Samuel Leung, Dmitry Turbin, Kelli Montgomery, Blake Gilks, Rob West, Melinda Carrier, David Huntsman, Samuel Aparicio

Dear Editor-in-Chief and Reviewers,

Thank you very much for considering our manuscript for publication in BMC Cancer. We have revised the manuscript according to the reviewers' suggestions. We believe that all comments have been addressed in a satisfactory manner and this revised manuscript now meets the standards for publishing in BMC Cancer.

Reviewer: 1

Major compulsory revisions:

1) “The authors’ should include comments about the fact that both machines will give rise to more FISH amplified cases to be scored as 1+ (negative). Add numbers for the drop off cases. How many for core drop-off, absent tumor etc.”

The suggested comment has been included in the last paragraph of the Discussion section. As for the numbers for the drop off cases and absent tumor, the Ariol system analyzes every picture, even if there is no actual core in it. When core is too small, partially damaged or missing, the Ariol still reports a score but the number of cells detected is usually less than 20. The cases with less than 50 cells detected (both positive and negative) were excluded from the statistical analysis, i.e. a filter was applied in the SPSS program. Thus unreadable cases included both hypocellular cores and missing cores. The pathologists also did not specify reasons for unreadable cores as similar data could not be available for the automated scoring.

Minor essential revisions:

1) “Add these authors to reference 12 as stated in Pubmed: Italian Network for Quality Assurance of Tumor Biomarkers (INQAT) Group; SIAPEC-IAP.”

Ref. 12 has been updated.
Reviewer: 2

Major compulsory revisions:

1) “Kaplan-Maier survival still needs explanation, since P values still appear unfeasibly low. There is possible confusion in the nomenclature – the lower case “e” might suggest natural logs rather than base 10 logs, which would make the P values more reasonable, but if so this needs to be clarified (the text uses upper-case “E” rather than “X 10x”. The end-point is still not clear - overall survival or breast cancer related death/recurrences?”

We specified in our previous cover letter that the end-point was breast cancer specific death and the p values were from log-rank test. This information has now been included in the Methods section.
The “E” on the P values reported represents “×10^x”. The P values are very small because of the large effects and the large sample size. We have attempted our Log Rank test calculations using different software (R and SPSS) and they give P-values of the same orders of magnitude if not the same P-value. All “E” notations in this manuscript have been changed to “×10^x” format to avoid this confusion.

Minor essential revisions:

1) “Full bibliographic details needed for Ref. 40.”

Ref. 40 has been updated.

2) “Table 6, middle row “FISH 1.8=2.2” should be “FISH 1.8–2.2”

Table 6 has been corrected.

(3) Criteria for unscorable FISH is only in terms of core degradation – need to include any criteria for inclusion based on minimum number of cancer cells, heterogeneous amplification, or morphological characteristics of tissue.

We explained in our previous cover letter that we only accepted scores if >40 tiles were counted. With Metafer system, one tile is considered 1 cell as the size of a tile is approximately the average size of a nucleus. Normal cells were excluded wherever possible, and the corresponding H&E slides were reviewed when needed. These criteria have now been included in the Methods section.

Language:

1. “(1) p. 17, fifth line from bottom, remove “, etc.”
This has been corrected.

Editorial comments

1) Unfortunately referee 2 did not receive your cover letter with the revised manuscript, please accept my sincere apologies for this oversight. We have since passed on your cover letter to the reviewer, however we have not received any additional comments. In order not to delay your manuscript further we can proceed on the basis of the comments we have received. Both the reviewers have raised additional minor points which we would be grateful if you could address. We appreciate that the answer to reviewer 2’s major point regarding the Kaplan-Maier curves was provided in your cover letter under point 8 of the response to referee 1. However, we believe that this point should also be clarified in the text of the manuscript.

This point has been clarified in the text.

2) Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

The revised manuscript conforms to the journal style.

Sincerely yours,

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