Author's response to reviews

Title: Triple-negative breast cancer is associated with EGFR, CK5/6 and c-KIT expression in Malaysian women

Authors:

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Version: 3 Date: 26 March 2012

Author's response to reviews: see over
Dear Sir or Madam,

26th Mar 2012

Enclosed please find the resubmission of the manuscript entitled “Triple-negative breast cancer is associated with EGFR, CK5/6 and c-KIT expression in Malaysian women” by Shant Kishen Kanapathy Pillai, Annie Tay, Suseela Nair, and myself (Manuscript number: MS 7183550976492164).

We wish to thank the editors and referees for their kind words and thoughtful comments. We agree with most of the comments by the reviewers and have adopted most of the suggested revisions in our paper. We also addressed the technical concerns raised by Reviewer #1, Reviewer #2 and Reviewer #3 by incorporating new information in the revised manuscript.

Thank you for your reconsideration of my work. Please address all correspondence concerning this manuscript to me at International Medical University, Malaysia and feel free to correspond with me by e-mail (cheeonn_leong@imu.edu.my).

Sincerely,

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Authors’ response to reviewers comments of the paper

“Triple-negative breast cancer is associated with EGFR, CK5/6 and c-KIT expression in Malaysian women”

(Manuscript: MS 7183550976492164)

Shant Kishen Kanapathy Pillai, Annie Tay, Suseela Nair and Chee-Onn Leong

Senior Editors’ comment:

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

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.

Authors’ response:

The authors wish to thank the reviewers for their time and thoughtful comments that led to a great improvement in the manuscript. We adopted most of the suggested revisions in our paper and addressed the concerns raised by the reviewers. We also revised and re-formatted the manuscript accordingly as to conform to the journal style
Response to Reviewer #1

Reviewers comment:

1) The authors should precise the criteria for patients inclusion in their cohort.

Authors’ response:

We thank reviewer #1 for reviewing our manuscript and pointing out the limitations and the ambiguities in our manuscript.

We agree that the precise criteria for patients inclusion are important and hence have made the changes accordingly in the materials and methods section.

Reviewer comment:

2) The authors state that specimens were primarily handled for ER PR and HER2 testing. They should describe the methods and precise the cutoffs used.

Authors’ response:

We agree and have made the changes accordingly in the materials and methods section. Negative for estrogen and progesterone receptors are defined as < 1% nuclear staining by immunohistochemistry. Negative for HER2 is defined as 0 or 1+ by DAKO Herceptest® Kit [1].

Reviewer comment:

3) The cohort of cases was obtained over a period of 2002 and 2006. Some technical variation could have occurred during this period that could impact on ER, PR and HER2 results. Was triple negativity of these cases re-established under standard conditions in the current study?

Authors’ response:

We have reviewed the protocols used in 2002 to 2006 and the conditions are essentially the same as in current practice. Therefore, we did not repeat the staining for ER, PR and HER2 in the current study due to the limited tissues that are available for this study.
Reviewer comment:

4) Did the authors include recent recommendations of using a 1% cutoff for ER and PR positivity?

Authors’ response:

Yes. We have adopted the 1% cutoff for ER and PR positivity and the cutoff is now being clearly defined in the materials and methods section of the revised manuscript.

Reviewer comment:

5) A table with Non-TNBC patient’s characteristics including ER, PR, and HER2 status should be added

Breast cancer is a heterogeneous group of disease. With a data base of 340 patients, it will improve the value of the study to match EGFR, CK5/6 and c-KIT staining and Ki67 index of TNBC with more subgroups of BC as RH+/HER2+, RH+/HER2- and RH-/HER2+.

Authors’ response:

We agree and included the characteristics of non-TNBC in Table 2 in the revised manuscript.

Reviewer comment:

p10 the sentence and references “as the percentage of Ki-67-positive nuclear profiles in randomly and systematically selected fields as described previously [18-20]” should be in the material and methods.

Authors’ response:

We agree and have added the statement in the materials and methods section.
**Response to Reviewer #2**

**Reviewer comment:**

*The paper is well written and the analyses well done;*

**Authors’ response:**

We thank reviewer #2 for his/her kind words and pointing out the limitations of our study for further improvements. The concerns raised by reviewer #2 are now being addressed as detailed in the following sections.

**Reviewer comment:**

1) When estimating the Ki-67 proliferation index you mention random and systematic sampling? can you explain further. Did you first select at random a starting zone and then systematically select a profile each given step.

**Authors’ response:**

For the Ki-67 proliferation index estimation, we first did a global observation to locate the cancer cells. Once we get a representative area, we then sampled each of the 200X viewpoint to make the cell counting until it reach about 500 cells.

**Reviewer comment:**

2) When you give means plus/minus a value specify if it is a standard deviation or standard error (even if it is very likely from the data that they are SD).

**Authors’ response:**

Since the data for Ki-67 proliferation index is based on cell counting, the values represent mean ± s.d.
Reviewer comment:

3) The author state that TNBC cases exhibit a higher frequency of p53 positivity suggesting that TNBC might associated with p53 misense mutation. This seems to me a strange conclusion since there is no evidence from the data that this is so. did the authors screened for p53 mutations?

Authors’ response:

We did not screen for the p53 mutation. However, based on the fact that p53 missense mutants are commonly stabilized and stained strongly by IHC, we speculate that tumors that are stained strongly with p53 are likely to be p53 mutated. This speculation is an underestimation as truncated mutants will stain negative for p53. Due to the ambiguity and confusion as pointed out by Reviewer 2, we decided to remove the statement from the manuscript.

Reviewer comment:

4) please give exact values of p-value up to one significant decimal and not just the upper bound.

Authors’ response:

We agree and have made the changes accordingly in the revised manuscript.

Reviewer comment:

5) what is the sens of UNKNOWN in table 1. do you mean undetermined?

Authors’ response:

We thank reviewer 2 for pointing out the ambiguity. Yes. It should be read as “undetermined”. We have made the changes throughout the revised manuscript and changed the “unknown” to “Not determined”.

Response to Reviewer #3

Reviewer comment:

Pillai et al. have studied TNBC in Malaysian women and have performed immunohistochemical analysis in subset of patients to evaluate basal-like markers. Although similar study may have not been done in Malaysian patients, the small number of patients included the IHC subset is a major shortcoming of the study. I also have following comments for improvement of the manuscript.

Authors’ response:

We thank reviewer #3 for reviewing our manuscript and pointing out the limitations and the ambiguities in our manuscript.

We agree that the major shortcoming of our study is the small number of patients included in the IHC subset. Of note, this is the first pilot study performed among Malaysian women and a bigger cohort is currently being planned. Nevertheless, our results are consistent and conform to the major observations reported in other population which also shown a close correlation between TNBC and CK5/6, EGFR and c-Kit [2-7].

Reviewer comment:

Previous Asian studies of triple-negative breast cancer including the one by Kim et al. (Hum Pathol 2006) should be included in the introduction and discussion.

Authors’ response:

We agree and have made the changes accordingly in our revised manuscript.

Reviewer comment:

It is unclear how HER2 negativity was defined. Had HER2 FISH been done in HER2 2+ cases?

Authors’ response:

The definition for HER2, ER and PR negativity has now been included in the Methods section of the revised manuscript.
Reviewer comment:

Please clarify if pathologist who specializes in breast oncology reviewed the IHC results. Please clarify if pathologist scoring IHC was blinded to other molecular profiles (triple negativity).

Authors’ response:

All IHC staining was evaluated by Assoc Prof Annie Tay, MBBS (Mal), MPath (UM), DRCPath (UK), FRCPath (UK) and confirmed by another in-house pathologist.

Reviewer comment:

Please clarify if pathologist scoring IHC was blinded to other molecular profiles (triple negativity).

Authors’ response:

All scoring of IHC was blinded. The specimens were collected, stained and made anonymous to the reviewers. The data collected were compared and compiled into the database.

Reviewer comment:

Please clarify how 36 patients for further IHC study were selected.

Authors’ response:

The 36 patients for further IHC study were selected based on the availability of the tissue samples in good condition and informed consents. All cases were age and grade matched as closely as possible.
Reviewer comment:

*The Ki67 index values are higher than those in previous studies, were adequate positive or negative controls included in the analysis.*

Authors’ response:

For Ki67 IHC staining, a mouse isotype-specific IgG was used as a negative control to determine non-specific binding of primary antibodies to human tissues antigen. In addition, we also used a MCF-7 and MDA-MB-468 cell block, and tonsil as positive control. Both MCF-7 and MDA-MB-468 cell block produce good staining of Ki67 while only the parabasal squamous epithelial cells of the tonsil show weak to moderate nuclear staining. Furthermore, the Ki67 produce adequate level of staining with negligible non-specific background in all the specimens tested, as shown in Figure 2. The reason for the higher Ki67 index than previous studies could be due to the fact that majority of the tumors used for IHC in this study were of high grade (grade 2 or 3).

We hope that the reviewers find that his/her concerns have been adequately addressed in the revised article, and in this response.

End of review.

Sincerely,

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References


