Author’s response to reviews

Title: Molecular essence and endocrine responsiveness of estrogen receptor-negative, progesterone receptor-positive, and HER2-negative breast cancer

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Author’s response to reviews:

Dear Senior Editor,

Please reconsider our manuscript "Molecular essence and endocrine responsiveness of estrogen receptor-negative, progesterone receptor-positive, and HER2-negative breast cancer" (MS#: 1470587730167927 and BMED-D-15-00216) for publication in BMC Med.

Thank you very much for reviewing our previous manuscript. We would also like to thank the reviewers for their thoughtful and constructive comments. According to the editor’s suggestions and the reviewers’ concerns, we have revised our manuscript and added new information where appropriate. We are pleased to resubmit our modified manuscript to you for further consideration.

Below, we include a point by point response to the reviewers’ concerns

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Editor’s comments
When making your revisions, please also provide a list of 3-10 keywords at the end of your abstract.

Our response:
Yes, we have revised it.

Advisor’s comments:

1. In general, the methods used to combine the cohorts (two-stage inverse-variance, fixed or random-effects used depending on heterogeneity) are now appropriate. But there are some concerns and/or more detail needed.

Our response:
Thanks very much for your suggestions.
2. In the first para of Results, phenotype proportions are pooled across studies. Caution is needed when pooling proportions; the use of standard methods may result in confidence intervals that include values outside [0, 1]. Examples of appropriate methods include arcsine transformations and Wilson confidence intervals; both are implemented by the user-written Stata program “metaprop”. The chosen method should be included in the “Statistics” section.

Our response:
In the revised manuscript, we used the command “metaprop_one” in Stata to calculate the pooled proportions. This method has clarified in the “Statistics” section.

3. The survival analysis (3rd para of Results) concerns three phenotypes and is therefore best analysed using multivariate meta-analysis. This may be done using the user-written Stata program “mvmeta” (or “network”, which is a more general version of the same method). This will give a pooled estimate of both ER-/PgR+ vs ER+/PgR+ and ER-/PgR- vs ER+/PgR+ simultaneously. Alternatively, two 2-way comparisons could be pooled independently, but these must then be simple splits of the data rather than removing one of the groups (otherwise bias may be introduced). In other words, e.g. ER+/PgR+ vs all others and ER-/PgR- vs all others. (Other parameterisations are also possible.) Currently, there are no pooled results in this section; only a verbal discussion of which cohorts reported a significant effect and which did not.

Our response:
According to the reviewer’s suggestion, two 2-way comparisons were pooled independently and the pooled survival estimates of ER-/PgR+ vs ER+/PgR+ and ER-/PgR- vs ER+/PgR+ were presented.

4. Furthermore, the “discussion” of this section (still 3rd para of Results) is written in terms of “validation” and reasons why Cohort 4 gives different results to the other cohorts. In terms of meta-analysis, it would be good to know the I-squared and (Cochran Q) p-value for heterogeneity here, to see how much evidence there actually is of Cohort 4 being meaningfully different from the others, especially in view of it having the smallest sample size of the four.

Our response:
Yes, we agree with the reviewer and have added the I-squared and (Cochran Q) p-value for between-study heterogeneity of the 4 cohorts.

5. Similarly, in the section “Intrinsic molecular subtypes…”, it is implied that Cohorts 3 and 4 were combined because they showed similar results, contrary to the philosophy of meta-analysis. Was relevant subtype data also available for Cohorts 1 and 2? If so, all four cohorts should be combined and displayed in Table 3 (and the commentary that Cohorts 3 and 4 showed similar, or the most interesting, results is then perfectly fine). If not, this should be clarified. (Also, see again my earlier comment on pooling proportions.) And again, was ESR1 data available for any cohort other than Cohort 4?

Please clarify.
Our response:

1. Because the relevant subtype data were unavailable for cohorts 1 and 2, we could pool only cohorts 3 and 4 together.

2. We actually combined the cohort 3 and 4 using a meta-analytical method rather than directly combined them. We used the command “metaprop_one” in Stata.

3. Moreover, the original data of ESR1 were only available in the cohort 4 and we could not analyze in the other 3 cohorts. We have clarified this.

6. In the section “Characterized gene expression…”, Mann-Whitney non-parametric tests are used on the data from all four (?) cohorts combined. Is there any chance that the expression levels could be transformed to (approximate) normality, so that pooled (meta-) analyses could be carried out instead?

Our response:
The original data of gene expression were only available in cohort 4 and we have clarified this situation. In this section, only the cohort 4 was analyzed and no meta-analysis was needed.

In the section “Refine the subtypes…”, it is stated that adjustment was made for other prognostic factors. Was cohort membership adjusted for? I would recommend that this be done even at the univariate (i.e. pre-adjustment) stage, to give a version of a one-stage fixed-effects meta-analysis. It should certainly be adjusted for along with the other prognostic factors, and its statistical significance noted.

Our response:
Thanks for your suggestion. In this section, we collected 64 consecutive cases with the ER-/PgR+/HER2- phenotype from FDUSCC between 2005 and 2011. These 64 cases were from FDUSCC rather than from the other 3 cohorts. It seems that it was not necessary to adjust for cohort membership for single-institute data. We have clarified this in the revised manuscript.

We hope that the editors as well as reviewers can satisfy our answers.

Thank you for your consideration.

Sincerely yours,

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