Author's response to reviews

Title: Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites

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Version: 4
Date: 27 November 2013

Author's response to reviews: see over
Author’s response to reviews

Title: Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites

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Version: 3 Date: 26 November 2013

Author’s response to reviews:

Dear editor,
Thank you for providing us the opportunity to improve this version of our manuscript with the comments and suggestions provided by the reviewers. Please find below in blue the responses to the comments raised in the review. In the revised text, the changes have been highlighted with track changes.

Yours sincerely,

Nynke Jager
Reviewer's report
Title: Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites
Version: 3 Date: 25 October 2013
Reviewer: Chi-Chen Hong

Reviewer's report:
The revised manuscript is much improved. There were just a few minor points given below.

• In table 2 it is not useful to provide median estradiol concentrations as <LL0Q. Would be better to provide the numbers and proportions for categories of estradiol levels: eg. <LLOQ, 43 to median among detectable values, above the median value among detectable levels. We changed the presentation of the estradiol concentrations in Table 2 as suggested by the reviewer.

• In supplementary table 4, instead of providing the range it would be better to provide standard deviations associated with metabolite levels. p-values can also be included even though they are not significant. We added the standard deviations to Supplementary 4.

• Would be helpful to include p-values in table 2, rather than just in the text. We included p-values in Table 2.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests
Reviewer's report

Title: Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites

Version: 3 Date: 16 October 2013

Reviewer: Thomas E Mürdter

Reviewer's report:

Comments on revised manuscript
Please try to use always the same format for pdf-files of the manuscript (preferable portrait not landscape). This would help to find changes / compare versions.

Minor Essential Revisions:

Table 1 + 2:
The following analyses were performed within 3 groups (pre-menopausal, post-menopausal + PTHF, post-menopausal without PTHF) and the only significant correlation between hot flushes and metabolites concentrations was observed in only one subgroup (post-menopausal + PTHF). Therefore, I recommend to present data in tables 1 and 2 accordingly in three groups.
We thank the reviewer for this comment. Since we are afraid this might compromise the overview of these tables, we prefer to present the data unchanged, however we added this additional table as an extra supplementary table (supp 6).

Line 169: (Supplementary 5).
Since supplementary table 5 is presenting results of our analysis, we prefer to refer to this table only in the results-section.
Line 173: multivariable analyses (supplementary 5).
Since supplementary table 5 is presenting results of our analysis, we prefer to refer to this table only in the results-section.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report

Title: Hot flashes are not predictive for serum concentrations of tamoxifen and its metabolites

Version: 3 Date: 19 October 2013

Reviewer: N. Lynn Henry

Reviewer's report:
The authors report associations between hot flashes and tamoxifen metabolites in 109 subjects.

Major compulsory revisions:
1. It remains difficult to understand how this study was conducted. It appears that patients were treated with tamoxifen as standard of care, and somehow they had blood samples drawn during treatment. In the first paragraph of the Patients and Methods section it states that they were determined as part of routine clinical care. However the first sentence under serum sample handling suggests that they were run in batch (not part of routine care). In the following paragraph it states that DNA was isolated from serum left over from the metabolite analysis, which also suggests the DNA isolation and genotyping was done in batch. The authors state that since this was an observational study with a single questionnaire they were not required to obtain IRB approval, but there is no mention of how they obtained patient consent for the blood draw or the genotyping.

We thank the reviewer for her time and effort to help us reporting our study more clearly. The most important is that we measure concentrations of tamoxifen and its metabolites as part of routine clinical care for our breast cancer patients on tamoxifen. These measurements are performed in batch every 4 to 6 weeks, so we can analyze more patient samples during one HPLC-MS analysis (which is time consuming and expensive) and the results are reported to the clinicians before the patients’ next visit to our clinic. We have more explicitly mentioned this in our manuscript by adding the following sentence to the paragraph Serum sample handling and determination of tamoxifen and metabolites “The patient serum samples were collected in serum gel tubes and stored at -70°C for some weeks, in order to analyze more patient samples during one HPLC-MS analysis.”

Another very important notice is the ethical concern about the patient consent. We already mentioned in our manuscript that for observational studies with a simple, single questionnaire it was not required to obtain approval of the Ethics Committee (Dutch Act on medical research involving humans, February 26, 1998), provided that there is compliance with Good Clinical Practice guidelines [23]. We now, in addition, explained this in the manuscript by adding the next sentence to Patients and Methods: “We performed this observational study with a simple, single questionnaire according to the national act on Ethics Committees (Dutch Act on medical research involving humans, February 26, 1998) and in compliance with Good Clinical Practice guidelines [23]. As a further interpretation of these GCP guidelines there is the “code of conduct of Human Tissue and Medical Research: Code of conduct for responsible use (2011)” by the Federa (http://www.federa.org/codes-conduct). In this code of conduct is stated that anonymous left-over body material may be used in observational clinical trials without explicit consent of the individual patients.”

2. Serum samples were collected between July 2008 and December 2011. When were the questionnaires completed? If all completed at one time after December 2011, it is unclear how patients could remember what was going on at the time their serum was drawn, since that could have been more than 3 years before. This would have a serious impact on the quality of the data, which is the key endpoint of the study.
We indeed realize this might be impacting our results and therefore we discussed this as a limitation.

3. What quality assurance was done for the genotyping? Were 10% of genotypes repeated to confirm the assay was working appropriately?
We added the following lines to the manuscript: “Genotyping was performed according to Standard Operating Procedures, using assays which were validated by direct sequencing. In each run, positive and negative controls were included.” and “The large percentage of genotyping failures can be explained by the fact that DNA was isolated from serum, which is a reproducible and validated method for genotyping in our lab, however the yield is low.”

4. How could missing CYP2D6 values be imputed with population medians? Were all patients with missing genotype assumed to be EMs? When 20% of the results are missing, and this is critical for the analysis, these assumptions could significantly impact the findings.
We agree with the reviewer that there is a possibility that this imputation may have affected the results. To address the issue we have included two sensitivity analyses for the multivariable analyses. In total three analyses are performed: one with the missing values imputed with EMs, one with the values imputed with P/IMs and one with these individuals excluded.
The association(s) between tamoxifen (and its metabolites) and the combined menopausal + hot flash history status is not affected by the choice of imputation of missing CYP2D6 values. Both the magnitude of the coefficients and the significance levels are unaffected. However when excluding these individuals we do see a loss of significance. Given that the magnitude of the coefficients does not change, we assume that this loss of significance is due to the exclusion of 18% of the sample, rather than a bias due to the imputation.

5. How were concomitant medications collected? It is difficult to believe that 64% of patients reported moderate-very severe hot flashes and none was being treated.
All individual patient records were checked by RHTK for concomitant medication. We found one patient who used medication to relieve hot flash complaints and excluded this patient from the analysis.

6. The final sentence of the conclusion still makes a statement that is not supported by evidence. It was not studied in this analysis and there are insufficient data in the literature to support this conclusion (only a single report, ref 8).
We agree with the reviewer that this statement might be a bit strong, when looking at the evidence that is reported so far. However, we added this sentence more as a recommendation for further research instead of an overall conclusion. Therefore, we moved this line from the Conclusion to the Discussion, and rephrased it, in order to provide an option for future research instead of a strong statement.

**Minor essential revisions**
1. P values should be listed in the tables (especially table 2)
We have added p-values in tables 1 and 2.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I declare that I have no competing interests