Author’s response to reviews

Title: Boronic Prodrug of 4-Hydroxytamoxifen is More Efficacious than Tamoxifen with Enhanced Bioavailability Independent of CYP2D6 Status

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Author’s response to reviews: see over
Dear BMC Cancer Editor,
Thank you for the opportunity to revise and improve our manuscript to address reviewers’ comments and suggestions. We have now completed the revision and have provided below a point-by-point response list for your review. All changes made to the manuscript text are highlighted in yellow for reviewers’ convenience.

Reviewer’s report
Title: Boronic Prodrug of 4-Hydroxytamoxifen is More Efficacious than Tamoxifen with Enhanced Bioavailability Independent of CYP2D6 Status
Version: 2
Date: 17 June 2015
Reviewer: Hitoshi Zembutsu

Reviewer’s report:
In this manuscript, authors showed that ZB497 effectively delivers a markedly increased plasma concentration of 4-OHT in mice and the boronic prodrug have far superior bioavailability compared to tamoxifen or 4-OHT as measured by the area under the plasma concentration time curve (AUC) using mouse model. However, the below critical issues were observed in this manuscript.

Major comments,
1. In result section (pp. 10-11), authors said that “While tamoxifen administration did not yield quantifiable endoxifen levels, 4-OHT orally given to mice generated an endoxifen concentration comparable to 4-OHT, with an AUC value of 61.2 ng/mL*h”. However, endoxifen has been known to reach greater than 6-fold higher plasma concentrations than 4-OH tamoxifen in human taking tamoxifen. If this discordance is due to the difference of species, the mouse model itself is not appropriate for this PK study.

Response:
We agree that tamoxifen metabolism in mice has many different characteristics from human metabolism. We are simply presenting the observation of endoxifen levels in mice blood.

We also agree and are aware that the endoxifen level is about 10-fold higher than 4-hydroxytamoxifen in breast cancer patients taking tamoxifen. We have added a statement in this paragraph for clarification of the metabolic difference between mouse and man (page 11). That said, the possibility cannot be fully excluded that ZB497 could generate significant levels of endoxifen in patients. However, this will only be found out in a phase I clinical trial which our research team is in the process of preparing for IND filing for FDA approval.

The mouse model has been used in numerous pre-clinical studies of tamoxifen metabolism and pharmacology, as illustrated in the following references:


2. In figure 4, 4 tumor growth curves (2 kinds of doses for tamoxifen and ZB497, respectively) except control looks similar. Was statistically significant difference observed between them?

Response:
Statistically significant difference was observed between the average tumor size of the mice administered with 0.1 mg/kg ZB497 and that of the mice given 0.1 mg/kg tamoxifen (p<0.05, now added in the text). In addition, the difference in tumor size between the 1mg/kg tamoxifen group and the 0.1mg/kg tamoxifen group was also statistically significant.

3. In figure 4, authors need to verify this results using several breast cancer cell lines.

Response:
We have previously tested the in vitro efficacy of ZB497 in both MCF-7 and T47D cells and have shown that the boronic prodrug acted as an anti-estrogen with similar potency in both cell lines. We agree that it would be desirable to validate the in vivo efficacy of ZB497 in a xenograft model based on other ER+ breast cancer cell lines, given that MCF-7 cells are by far the most widely accepted and used xenograft model for ER+ breast cancer, we believe that it is sufficient to use the MCF-7 xenograft model for the scope of work presented in this manuscript.

4. Although I do not know how the authors calculated, the following description in discussion section (p. 15) is inappropriate. This should be deleted.

“While it is difficult to make a direct comparison between human and mouse dose responses, it should be pointed out that the human dose for tamoxifen varies between 20 and 30 mg/day, which depending on patient weight would be in the range of 0.2-0.3 mg/kg. Thus, the 1 mg/kg dose we used would roughly correspond to 3-5 times the standard human dose.”

Response: We have now deleted this paragraph in the manuscript.

Level of interest: An article of insufficient interest to warrant publication in a scientific/medical journal

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests: I declare that I have no competing interests

Reviewer's report
Title: Boronic Prodrug of 4-Hydroxytamoxifen is More Efficacious than Tamoxifen with Enhanced Bioavailability Independent of CYP2D6 Status
Version: 2
Date: 2 July 2015
Reviewer: Anna Herman-Antosiewicz
Reviewer's report: This is a well written paper showing very interesting results on pharmacokinetics and bioavailability in mice of boronic prodrug developed to deliver active form of tamoxifen, 4-OH-tamoxifen. Presented results show its superiority over 4-OH tamoxifen and tamoxifen, as it reveals better bioavailability, even in low concentrations provides much higher plasma concentration of active tamoxifen metabolites, and their higher accumulation in tumor tissues.
Specific comments for the major compulsory revision: 1. Authors should show or at least mention the possible side effects of the tested pro-drug (overall body weight, effect on uterus, etc) in comparison to drugs
already in use.

Response:
We agree. Additional discussion is now included in the Discussion section regarding possible side effects of ZB497. In particular, we included discussions and relevant references regarding the byproducts of ZB497 as it is converted to 4-hydroxytamoxifen under physiological conditions (see page 16).

2. Authors should reevaluate t1/2 values, as these presented in Table 1 do not match with Fig. 3D (especially 4-OHT and ZB497). Besides, data presented in Fig.3 seems to refer to just one experimental animal (no SD provided); Y axis for tamoxifen (Fig.3D) is incorrect. The clearance time for the new drug should be commented in relation to tamoxifen or 4-OH tam.

Response:
We thank the reviewer for pointing out this important discrepancy due to our negligence. We have recalculated the t\textsubscript{1/2} values using the PK software with manual corrections for the data points for more accurate regression curves. The corrected t\textsubscript{1/2} values are now included in Table 1.

The data presented in Figure 3 were based on the mean plasma concentration of 3-5 mice blood samples depending on the time point (each time point involved at least 3 mice for blood sampling). We have now included error bars that are calculated based on standard errors.

We have included comments on the clearance time for ZB497, tamoxifen, and 4-OHT observed in mice administered by oral gavage. (See page 15 in the discussion section)

Specific comments for minor essential revisions
1. Figure legends should be written in more detail,

Response:
We have included more details in the Figure legend where appropriate

2. Last sentence of Introduction: should be “with” instead of “of”

Response:
This has been corrected.

Specific comments for discretionary revisions
1. Fig.2- It would be helpful to present the amounts of respective metabolites (which is mentioned in the main text) in the figure

Response:
The amounts (%) of respective metabolites are now included in Figure 2.

Level of interest: An article of outstanding merit and interest 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