Author's response to reviews

Title: Effects of liarozole fumarate (R85246) in combination with tamoxifen on N-methyl-N-nitrosourea (MNU)-induced mammary carcinoma and uterus in the rat model

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Dear Dr. Pemberton,

MS ID: 9236076781038909
Effects of liarozole fumarate (R85246) in combination with tamoxifen on N-methyl-N-nitrosourea (MNU)-induced mammary carcinoma and uterus in the rat model
Paul E Goss, Kathrin Strasser-Weippl, Shangle Qi and Haiqing Hu

We are very grateful for the useful remarks, questions and suggestions offered by your reviewers of our manuscript. We have carefully addressed these in the order in which they were presented to us and have outlined our responses below.

We have revised our manuscript and ensured the revised version conforms to the journal’s style and format. We hope that the changes we have made, and the explanations offered, will be satisfactory to the reviewers and the BMC Editorial Team. We believe that our manuscript has been significantly improved with your advice and help. We look forward to hearing from you.

Yours sincerely,

Paul E. Goss, MD, PhD, FRCPC, FRCP(UK)
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Director of Breast Cancer Research, MGH Cancer Center
Co-director of the Breast Cancer Disease Program, DF/HCC
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1. Comments from Reviewer Dr. Henry J Thompson

Comment (1)
While the topic of this manuscript is of interest, there are apparent inconsistencies between what is described in the Methods section and the information provided in the legend of Figure 1 that make it difficult to interpret the study findings. Specifically, were animals in the tumor growth study ovariectomized as indicated in the legend of Figure 1? If they were, what is the rationale for expecting tamoxifen to have an effect on tumor growth? Similarly, what is the rationale for expecting an aromatase inhibitor to work in an ovariectomized rat since it does not appear that rat adipocytes have aromatase activity; the authors should indicate where they expect the aromatase inhibitor to be exerting its effects. Other concerns are detailed below:

RESPONSE (1)
The reviewer is correct. The MNU-induced rat mammary carcinoma was growing in intact, not ovariectomized, female Sprague-Dawley rats. We have made the correction to the legend of Figure 1 as indicated below:

“Figure 1 Effects of liarozole alone and in combination with tamoxifen on tumor burden of MNU-induced rat mammary carcinoma in cycling female Sprague-Dawley rats.”

Comment (2)
The data shown in Figure 1 appear to be smoothed growth curves. It would be more informative to show the actual data with error bars graphed.

RESPONSE (2)
The data shown in Figure 1 appear as linear square root tumor burden (mm), because a two-stage linear modeling approach for the analysis of repeated measures was used for the primary analysis (Ref. 1) and an auto-regressive covariance structure was used to model the square root transformation of the observed data (Ref. 1) in the tumor burden experiments. Using this method it is correct not to show error bars and p values are recognized as sufficient to demonstrate significance.

However, in accordance with the suggestion of the reviewer, we have shown actual data – the average tumor burden area at the end of the treatment period, as well as p values in the ‘Results and Discussion’ section as follows.

“The mean tumor burden for each treatment group is shown in Figure 1. At the end of the treatment period, the average tumor burden area of the L20 (525 mm²), L80 (452 mm²), L20+T100 (117 mm²), L80+T100 (84 mm²) and T100 (32 mm²) groups, was significantly smaller than in the control animals (1375 mm²) (all p values < 0.05).”

Comment (3)
While brevity has merit, there is essentially no discussion of the data presented, and the discussion that is presented is vague. The lack of discussion implies that the findings have limited importance.

**RESPONSE (3)**
At the suggestion of the reviewer we have extended the discussion section, addressing all the reviewers’ specific questions. We still believe that presenting the data and its implications in a concise and clear way is of value to the oncology audience and in elaborating on the implications of our results we have nevertheless striven to avoid extending the length of the paper unnecessarily.

**Comment (4)**
*What do the authors think is the basis of liarozole’s apparent reduction of tamoxifen’s effect on the uterus?*

**RESPONSE (4)**
The retinomimetic effect of liarozole likely overcomes the estrogen agonist effect of tamoxifen on the uterus - in a similar manner other retinoids have been shown to reduce the effects of estrogen on the uterus.

We have added a sentence in part two of the results section pointing out the antagonistic effects of the combination of tamoxifen and liarozole on the endometrium.

**Comment (5)**
The authors use a very vague term, anti-tumor to describe the activity of liarozole. This could be misleading to some readers. The compound slowed tumor growth rate; it did not induce tumor regression and it appeared to antagonize the effects of tamoxifen. Only tamoxifen induced tumor regression, which is the desired outcome.

**RESPONSE (5)**
We have now stated more clearly in the ‘Results and Discussion’ section that only tamoxifen lead to tumor shrinkage whereas liarozole merely stopped tumor growth. Although tumor regression is the desired outcome in established breast cancer, in chemoprevention preventing tumor growth and minimizing side effects are as important. In this setting, the combination of a retinoid with tamoxifen might be of merit. We have now modified the abstract’s conclusion to point this out more clearly.

**Comment (6)**
*Since liarozole did not induce tumor regression and antagonized the effects of tamoxifen, the conclusion stated in the abstract is too strong.*

**RESPONSE (6)**
The term “efficacy” was replaced by “clinical value” in the conclusion of the abstract in order to point out that the goal of combining a retinoid with a SERM is not necessarily to improve efficacy but rather to reduce toxicity.

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2. Comments from Reviewer Dr. Vincent Njar
Comment (1)
This manuscript by Goss et al. describes clearly and thoroughly the effects of liarozole fumarate in combination with tamoxifen on N-methyl-N-nitrosourea (MNU)-induced mammary carcinoma and uterus in the rat model. The findings that liarozole did not decrease the anti-tumor effect of tamoxifen in contrast to the combination of tamoxifen with anastrozole (a pure aromatase inhibitor) and also the demonstration that liarozole is able to reduce undesirable uterotrophic effects of tamoxifen is very important. The results of this study are likely to provide the rationale for clinical studies of SERMs in combination with RAMBAs. The work should capture the interest of the wide breast cancer drug development and oncology audience, and it is timely.

RESPONSE (1)
We thank the reviewer for recognizing the importance of our findings. We agree that the finding that liarozole did not decrease the anti-tumor effect of tamoxifen in contrast to the combination of tamoxifen with anastrozole or letrozole, and also the demonstration that liarozole is able to reduce undesirable uterotrophic effects of tamoxifen are of particular importance.

We believe the results of this study provide the rationale for further clinical trials of SERMs in combination with RAMBAs, and as such are of significant potential importance to produce more effective and better tolerated therapy for the treatment and prevention of breast cancer. RAMBA’s continue to be of importance in breast cancer research as indicated by a paper presented at the recent 7th International Aromatase Inhibitor meeting hosted by the University of Maryland in Baltimore.

Comment (2)
I suggest a few changes:
2. Figure 1: The authors should indicate all average tumor volumes in the graph.

RESPONSE (2)
1. We thank the reviewer for suggesting a number of important additional references and we have cited those applying to RAMBAs in our “Background” section.

2. The data shown in Figure 1 appeared as linear square root tumor burden (mm), because a two-stage linear modeling approach for the analysis of repeated measures was used for the primary analysis (Ref. 1) and an auto-regressive covariance structure was used to model the square root transformation of the observed data (Ref. 1) in the tumor burden experiments.

According to your suggestion, we have shown actual data - average tumor burden area at the end of the treatment period, as well as p values in the ‘Results and Discussion’ section as follows:
“The mean tumor burden for each treatment group is shown in Figure 1. At the end of the
treatment period, the average tumor burden area of the L20 (525 mm²), L80 (452 mm²),
L20+T100 (117 mm²), L80+T100 (84 mm²) and T100 (32 mm²) groups, was
significantly smaller than in the control animals (1375 mm²) (all p values < 0.05).”

3. Comments from Reviewer Dr. Konstantin Christov

Comment (1)
Liarozole fumarate (R82246) alone and combined with tamoxifen has been used to
suppress the progression of established mammary tumors in rats. Liatrozole is an
aromatase inhibitor which is no more in the market. Its combination with tamoxifen is not
appropriate because they work on ER+ mammary tumor cells by similar mechanisms,
therefore the results on tumor burden are not additive.

RESPONSE (1)
Liarozole is no longer on the market, but both aromatase inhibitors and retinoids are
available. Therefore, this study can be seen as a proof of concept that retinoids reduce
tamoxifen-induced toxicity without reducing its anti-tumor effects. We have now added a
sentence pointing this out at the end of the ‘Results and Discussion’ section.

As stated in paragraph 3 of the introduction, retinoids and aromatase inhibitors do not
work by the same mechanism of action on ER+ tumor cells.

As mentioned above RAMBA’s continue to be of interest to researchers looking for
novel ways to improve breast cancer treatment and prevention. Our results support these
efforts.

Comment (2)
The subcutaneous injection of tamoxifen is not the appropriate pathway for testing the
efficacy of various doses.

RESPONSE (2)
Giving tamoxifen to patients by the oral route is current clinical practice. While it is true
that tamoxifen has also been given by oral gavage to experimental animals (rats and
mice), subcutaneous injection of tamoxifen continues to be widely used and accepted for
animal studies and we have cited a number of references indicating this (Ref. 2 – 17).

Comment (3)
From the slides presenting the alterations in the endometrium it appears that apoptosis is
strongly involved in mediating the effect of both agents.

RESPONSE (3)
The slide shown in figure 3 indicates that the inhibitory effect of liarozole significantly
negates tamoxifen’s stimulatory effects on the endometrial epithelial cells. The slide
shown in figure 4 indicates that the strong inhibitory effects of liarozole on the
endometrium significantly negate tamoxifen’s agonistic, proliferative effect on the
endometrium. These anti-estrogenic effects of liarozole on the endometrium might be
worth exploiting in endometrial cancer treatment or even prevention. We agree that the
alterations in the endometrium appear related to apoptosis. Published data suggest that
the increased proliferation induced in the endometrium by tamoxifen alone is not offset by enhanced apoptosis (Ref. 18) and we must therefore attribute the apoptosis highlighted by the reviewer to be due to the effects of liarozole.

REFERENCES


12. Son HY, Nishikawa A, Okazaki K, Lee K, Imazawa T, Hirose M: Lack of modifying effects of atrazine and/or tamoxifen on thyroid carcinogenesis in rats pretreated
with N-bis(2-hydroxypropyl)nitrosamine (DHPN). *Food Chem Toxicol* 2003, **41**:1811-1816.


