Author's response to reviews

Title: Targeting glycolysis by 3-Bromopyruvate improves Tamoxifen Cytotoxicity of Breast Cancer Cell Lines.

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Author's response to reviews: see over
The ARRIVE Guidelines Checklist
Animal Research: Reporting In Vivo Experiments
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<table>
<thead>
<tr>
<th>ITEM</th>
<th>RECOMMENDATION</th>
<th>Section/Paragraph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title</td>
<td>1</td>
<td>Provide as accurate and concise a description of the content of the article as possible.</td>
</tr>
<tr>
<td>Abstract</td>
<td>2</td>
<td>Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Background | 3 | a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. 
   b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study’s relevance to human biology. | We hypothesized that the use of glycolytic inhibitor (3 BP) could increase TAM efficacy on MCF-7 and T47D cell lines, as well as on mice bearing Ehrlich solid tumor. |
| Objectives | 4 | Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. | |
| METHODS | | | |
| Ethical statement | 5 | Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal (Scientific Procedures) Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. | All of the animal handling and study procedures were approved by the research ethics committee of Faculty of Pharmacy, Cairo University, Cairo, Egypt (Permit Number: PT 661), and was conducted with the "Guide for the Care and Use of Laboratory Animals" |
a- Ehrlich carcinoma (EAC) cells (2 x 10^6) were transplanted subcutaneously in the right thigh of the lower limb mice. Mice with a palpable tumor mass (approximate 100 mm^3), which developed within 7 days after implementation.

b- We divided the animals randomly and blindly into 4 groups each of 6 animals.

Group one injected i.p with 5mg/kg TAM, group two injected with 3-BP (10mg/kg i.p), group three treated with their combination and control group received saline. Treatment continued twice/weekly for 3 weeks. The procedure was done in the animals' facility in National Cancer Institute, Egypt.

Animals were anesthetized with sodium pentobarbital 100 mg/kg i.p was used then cervical dislocation with high degree of proficiency to anesthetized animals according to Euthanasia guidelines.

a- Female Swiss albino mice, weighing 22-25 gm, were obtained from the National Cancer Institute, Cairo, Egypt.

b- Not apply as we do not knock out or transgenic ……

Not applicable we do not use fish.

- The total mice 24 per experiment (6 mice for 4 groups) and the number used for the tumor volume experiment: 6 mice for each group; for oxidative stress markers: 6 mice and for IHC: 3 mice for each group.

- The sample size was chosen to be able to make standard deviation and statistics.

a- Randomly.

b- First the 3-BP was given to mice then Tamoxifen to the next group and finally the combination was given to fourth group.

Treatment continued twice/weekly for 3 weeks. The change in tumor volume was measured using venire calibre and calculated by the following formula according to Osman et al, [22].

**Immunohistochemistry VEGF**
RESULTS

The mean of weight = 22-25 gm.

The control group in this study is the mice bearing Ehrlich tumor model.

The results of the control and treated groups are presented as histogram and figures in the manuscript.

DISCUSSION

The results of in vivo was in harmony with in vitro ones and the combination showed better effect on the tumor volume, oxidative stress and VEGF.

Ehrlich model is a murine mammary tumor model and it was better to use nude mice bearing MCF-7 or T47D cells which are not applicable in NCI.

Using 3D tissue culture may reduce the need of animals but it is not available in the lab.

This study is a preliminary preclinical one that should be followed by more investigations.

No fund.

The animals were brought from the animal house of NCI.