**Rebuttal Letter**

Manuscript # JoVE58291 "An effective method to investigate the synergism between anti-cancer antibodies and chemotherapeutic drugs in vitro and in mice."

Authors: Bahri et al.

**Editorial and production comments:**  
  
Changes to be made by the Author(s) regarding the written manuscript:  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.  
**Reply:** Done.

2. Please revise lines 69-72, 389-391, 392-421, 484-486, 488-489, 494-497, and 559-562 to avoid previously published text.

**Reply:** Done.

3. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

**Reply:** Done.

The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

**Reply:** Done.

4. Figure 1: Please change “ml” to “mL”.

**Reply:** Done.

5. Figure 2: Please ensure that the panels are of the same dimensions if possible.

**Reply:** Done.

6. Figures 5 and 6: Please change “sem” to “SEM”.

**Reply:** Done.

7. Please revise the title to be more concise.

**Reply:** Done. The revised title is “Potentiation of anti-cancer antibody efficacy by anti-neoplastic drugs: detection of antibody-drug synergism using the combination index equation.”

8. Keywords: Please provide at least 6 keywords or phrases.

**Reply:** Done. We provided the 9 following keywords: cancer research; drug development; antibody-drug combination; antibody-drug interaction; MTT assay; antibody-drug synergy; combination index equation; *in vitro* cell line model; tumor xenograft model.

9. Please rephrase the Abstract to more clearly state the goal of the protocol.

**Reply:** Done.

10. Please define all abbreviations before use.

**Reply:** Done.

11. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "CompuSyn" and “Matrigel” within your text. The terms may be introduced but please use them infrequently and when directly relevant. Otherwise, please refer to the terms using generic language.

**Reply:** Done. After introducing the terms Matrigel and Compusyn we replaced “Matrigel” by basement membrane matrix and “Compusyn” by “simulation software”.

12. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.”

**Reply:** Done.

Please include all safety procedures and use of hoods, etc.

**Reply:** Safety procedures are reminded at the beginning of the Protocol Section.

Please move the discussion about the protocol to the Discussion.

**Reply:** Done.

13. Please revise the Protocol steps so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary.

**Reply:** Done.

14. 1.1.3: What is used to count cells?

**Reply:** We used a hemocytometer. This is indicated in the revised manuscript.

15. 1.1.6: Please specify other incubation conditions (temperature, %CO2).

**Reply:** The temperature condition is 37°C and the % of CO2 is 5%, as added in the revised manuscript.

16. 1.3.4: What is the humidity?

**Reply:** 95 %, this has been in the revised manuscript.

17. 2.1.3: What happens after centrifugation? Is the supernatant discarded?

**Reply:** The supernatant is discarded, as corrected in the revised manuscript.

18. Please ensure that the computational steps of the protocol are done in a graphical user interface with explicit user input commands: File | Save | etc.

**Reply:** Done. We have re-written the corresponding Section.

19. Please revise to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc.

**Reply:** Done. The Representative Results Section has been re-written accordingly.

20. References: Please do not abbreviate journal titles.

**Reply:** The full journal titles are now indicated.

21. Table of Equipment and Materials: Please provide lot numbers and RRIDs of antibodies, if available.

**Reply:** Antibody 8B6 was generated in our laboratory. Lot numbers and RRID are not available.  
  
Changes to be made by the Author(s) regarding the video:  
  
1. Please increase the homogeneity between the written protocol and the narration in the video. It would be best if the narration is a word for word from the written protocol text.

**Reply:** We edited the video and homogenized the narration and the written protocol text.

2. Titles of the manuscript and the video do not exactly match.

**Reply:** Correct. Done, thanks.

3. The details in the video are not the same as the details in the written manuscript. For example:  
03:13: The video says incubate overnight while the written manuscript states 18 h.

**Reply:** Correct. Done, thanks.

04:15: Please use the same figure in video and in the written manuscript.

**Reply:** Done.

04:24: The video says 38 °C incubate overnight while the written manuscript states 37 °C.

**Reply:** Correct, fixed, thanks.

4. 04:54: Please remove commercial language from the video (Excel).

**Reply:** Done.

5. Please upload a revised high-resolution video here: <http://www.jove.com/files_upload.php?src=17778803>

**Reply:** Done.

6. Audio issues  
• The audio volume of the narration is a bit low. To match with the other videos on our site, the audio levels should be peaking between -6 and -12 dB.

**Reply:** Fixed.

• 9:57 - It sounds like the audio fades out while the narrator is still speaking. This should be corrected.

**Reply:** Fixed.  
  
**Reviewers' comments:**  
  
  
**Reviewer #1:**  
This is a straightforward and well-written manuscript. But Bahri et al unable to evaluate the outcome of this study by using any standard approach.

**Reply:** The outcome of the study has been previously published by our group as cited in the manuscript. Here we focused on the Chou and Talalay method.   
  
Some suggestions for the authors' consideration:  
1. The title of this manuscript is not suitable and did not provide any impact of the outcome of this study. It is hard to get away from that fact. Please improve it.

**Reply:** Done. The revised title is “Potentiation of Anti-Cancer Antibody Efficacy by Anti-Neoplastic Drug: Detection of Antibody-Drug synergism using the Combination Index Equation.

2. It would be good if authors add a schematic representation of this study.

**Reply:** We added a schematic representation of the study (Fig. 1) is the revised manuscript.

3. Please mention any standard approach using FACS/ Western blot analysis or Immunohistochemistry to prove the hypothesis.

**Reply:** Good suggestion. This has been added in the Discussion Section. Thanks.

4. Please also check the survival rate after treatment.

**Reply:** Fair point.Done, see Fig. 5B. Thanks.

5. Protocol for Antibody and drug preparation is too long and ambiguous. Author can easily mention that the used serial dilution of compounds/ Antibody rather than to repeat about the dilution.

**Reply:** We have shortened protocol Section accordingly.

6. Discussion- Please also correlate the outcome of this study with references from the other published studies in large cohort.

**Reply:** I am afraid but the combination regimen has not been tested into patients yet.  
  
  
**Reviewer #2:**  
Major Concerns:  
The authors correctly mention that the 8B6 monoclonal antibody can kill tumor cells through either antibody-dependent cell mediated cytotoxicity or through apoptosis. However, the assay described by the authors does not measure apoptosis directly (MTT assay measures cell viability, and xenograft assay measures tumor size). The authors need to demonstrate that the cells are being killed, and the effect is not just cytostatic, and is not simply a result of cell proliferation decrease due to combination drug load. This is particularly important because the xenograft experiment is in immunodeficient mice, so it is unlikely the tumors are being killed by antibody-dependent cell mediated cytotoxicity. A simple immunohistochemistry for apoptosis markers such as cleaved caspase-3 on xenograft tumors should be sufficient to demonstrate this. Alternatively, Annexin V staining in cell lines treated with the drug combinations should be able to show that the cells are being synergistically killed if the author's conclusions are correct.

**Reply:** We agree with you. MTT assays only assess the cell viability. In addition, the killing mechanisms of mAb 8B6 have been already published as cited in the Discussion Section. This is clearly stated in the Discussion Section of the first submission. In respect with your comment, we added in the revised manuscript Discussion Section the list of the standard methods we used, in our previously published works, to assess apoptosis *in vitro* and in tumor xenografts biopsies.

Minor Concerns:  
-In line 134 the authors mention using 0.05% EDTA solution to dissociate cells. I believe the authors meant to say Trypsin-EDTA solution. Please clarify

Good question. However, there is no typo. 0.05% EDTA solution can dissociate cells such as IMR5 cells. The use of a trypsin-EDTA solution would resulted in a decrease of the cell viability since these cells are very sensitive to trypsin.

-In line 416 the authors need to mention which statistical test was used to calculate the p values

Good suggestion. Done. Thanks

-Figure 2A the authors mention tail vein injection, however the figure indicated shows intraperitoneal injection. Please clarify and use appropriate image

Thanks. We have modified Fig. 2 to match the Figure legend.

-Figure 3 the authors need to include statistics

Good suggestion. Done. Thanks