Author's response to reviews

Title: In vitro and in vivo anticancer properties of a Calcarea carbonica derivative complex (M8) treatment in a murine melanoma model

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Author's response to reviews: see over
Curitiba, 2009, December, 08.

Editor

BMC Cancer,

Dear editors Dr Sabina Alam & Dr Erik Alexandersson,

Enclosed you will find the actualization of the manuscript entitled “In vitro and in vivo anticancer properties of a Calcarea carbonica derivative complex (M8) treatment in a murine melanoma model” by Fernando de S. F. Guimarães, Lucas F. Andrade, Sharon T. Martins, Ana Paula R. Abud, Reginaldo V. Sene, Carla Wanderer, Inés Tiscornia, Mariela Bollati-Fogolin, Dorly de F. Buchi and Edvaldo S. Trindade as myself being the corresponding author. All authors are aware and support the submission of this manuscript as authors. The follow modifications or observations were done as Reviewer`s suggestions and observations.

Hoping to hear from your soon,

Yours sincerely,

Edvaldo da Silva Trindade, PhD
Professor of Cellular Biology
Curitiba, 2009, December, 08.

Dear Dr Sabina Alam & Dr Erik Alexandersson

We are returning the manuscript with correction of pertinent observations. Also we are thankful by the rich suggestions. The manuscript was revised. The part of Figure 6 was combined to Figure 5 and the other Figures were renumbered. Finally the manuscript was submitted for correction in an English editing service (http://www.journalexperts.com/). The text bellow shows a list of reviewer’s comments followed by our answer.

Reviewer #1 (Gitte Jensen):

- “Page 12, line 22: "xilene" should be "xylene"." – **OK, done.**
- “Page 13, line 19: "polimerization" should be "polymerization"." – **OK, done.**
- “Page 14, lines 19-21: This is a very important observation, and you should provide the P value, even if it did not reach statistical significance. The sentence needs to be re-worded. For example: Cell cycle was evaluated and a clear tendency (or trend) to a decrease in the number of cells in S and G2 stages was observed; however, the data did not reach statistical significance (p=...).” – **OK, the sentence was updated according the good suggestion.**
- “Page 15, line 18-19: change beginning of the sentence to: "A decrease in the number of tumoral nodules was observed..."” – **OK, done.**
- “Page 16, line 12-15: The sentence is unclear - please reword.” – **OK, done.**
• “Page 16, line 16: remove the 's' from the end of the word mitochondria. (The word mitochondria is plural without the 's').”  – OK, done.

• “Page 16, line 17-18: Change sentence to: "Furthermore, many dying parenchyma cells with picnotic nucleus were seen...””  – OK. Thank for observation. The paragraph was changed.

• “Page 17, Line 18: Change to: "After M8 treatment, perlecan expression was significantly down regulated in tumor nodules...””  – OK, done.

• “Page 18, line 17: Start sentence: "Progression of melanoma, as well as other malignant cancers, ...””  – OK, done.

• “Page 20, lines 3-6 are unclear. Try: "...but there was a clear tendency for --- to be close to the levels seen in normal mice...””  – OK, done.

Because the manuscript was revised and submitted an English editing service, the lines mentioned above could be not represent in the new version, but all suggest were accepted.

Reviewer #2 (Hiroaki Sakurai):

• “The results in Fig. 3. do not show the anti-metastatic activity of M8. The statistical test should be tested in group c (vehicle) vs d (M8 treated).”  – OK, an additional comparison between groups C and D was performed and added in Fig 3E, as well as in its figure legend. The difference was still significant *P << 0,05 after comparison between M8 and treatment.

• “Fig. 2: It is necessary to count the cell number in at least three fields and the data should be tested the significance.”  – The MEV figures are a representative of each group. The ImageJ analyses was performed only the figure shown. Then we added in the text the follow sentence: “These data suggest that M8 decreases cellular invasion.” (page 15, last line of the first paragraph).

Reviewer #3 (Hua Wang):
“Since the authors don’t think C57BL/6 mice suitable for immunological questions study, they should use another animal model to confirm their experiments.” The focus of the present study wasn’t the immunologic evaluation but the effects of the treatment in a malign model of melanoma. According the Current Protocols in Immunology this is the best model of metastatic melanoma in mice because reflects several parameters as the human melanoma. Additionally, there is no published studies using this specific cell lineage (B16F10) in other mice strain.

“The authors didn’t do any experiments that can support their conclusion (these findings suggest that this medication is a promising combination-therapy candidate).” – OK, the conclusion was changed to: “These findings suggest that M8 is a promising therapy that may improve the innate immune response against tumor cells, decreasing the cell adhesion or invasion of malignant cells or even inducing direct dormancy in malignancies. Further studies are necessary to clarify the precise and detailed mechanism of M8 treatment in anti-tumor effects in melanoma and its use as combination-therapy candidate used in addition with conventional medicines”

The paper is poorly written and has many misquotes of literature results. For example: Page 7 line 11-12“All in vitro experiments were performed at 37ºC in a humidified atmosphere containing 5% CO2 during for 48 h. Page 19 line 21-22 “it is the most used murine model to simulate metastatic melanoma because reflects several characteristics of human metastatic melanoma” – Thanks for these observations. The literature was scrutinized revised and fixed. The manuscript was revised and text was submitted a English editing service (http://www.journalexperts.com/).

“What Y axis represents in Figure 3, 7, 9, 10 is not shown.” – OK, a meaning of Y axis was added in graphs.

“Figure9: The SD is doubtful in "GR+"cells.” - We agree that The error is big but no significant differences were found among all animals tested.
• “In vitro reporter cells (MxRAGE7 and HT29-pNF-#B-hrGFP) experiments are missing. These experiments had nothing to do with cell adhesion or invasion.” – These are screening experiments used here in way to amply action mechanism detection in some tumor response associated parameters. Because this data were place in supplementary data.

Reviewer #4 (Haili Qian):

• “The author used lung inhalation as a way to stimulate the immune system to suppress the lung metastasis of melanoma cells. To exclude the direct suppression of the M8 complex on the tumor growth, it is better to administer the M8 complex by other ways but not lung inhalation to confirm the immune effects of M8 on metastasis. Or try the effect of lung inhalation on the subcutaneous tumor model.” - The inhalation treatment was elected because it distributes the medication directly in animal lungs, the specific metastasis site. Additional, this kind of treatment is very commonly medically prescribed to patients in complementary medicine in clinics to be less aggressive and painful as intravenous or even intramuscular.
• “Please include the methods to count the number of lung metastatic lesions.” – OK, a description of the method was added in Materials and Methods - B16F10 lung metastasis and in vivo treatment, pg 11, lines 2-4.
• “Please include the statistics the authors used in the Methods section.” – The used statistics are placed in the last topic of methods section: Micrographs and statistics analysis. Page 14.