I have read with interest the paper by Almeida and coll, that is focused on the effects of \( \omega-3 \) and \( \omega-6 \) against melanoma growth. The experiments have been conducted both in cell cultures as well in murine model (C57BL/6 mice) and the effect on cytokines (IL-6, IL-10 and CXCL-1) and on inflammatory mediators (Leucotrienes and prostaglandins) have also been evaluated.

Unless the research methods are adequate and well discussed, I believe that the effect of fatty acids has not been properly investigated: as it is stated in the Materials and Methods section, the effect of \( \omega-6 \) alone has not been evaluated. Hence, the experiments were conducted by the use of: i) fish-oil rich in \( \omega-3 \), ii) soybean oil rich \( \omega-6 \), but containing both \( \omega-3 \) and \( \omega-6 \), and iii) a mixture of fish-oil and soybean oil in 1:1 ratio. On this basis, even when only soybean was administred in cell culture media or injected in mice, it is not composed only by \( \omega-6 \). Since the effect of the mixture reached the best effect against melanoma growth, one can argue that the effect could be due to the \( \omega-6 \) alone. How can we sustain that the obtained results are due to the mixture or just to the \( \omega-6 \)? This is an important point to clarify, and further experiments should be performed. In case this would not be possible, I believe that clearly explained in the text.

Author response: Thank you so much for these comments. We would like to mention that although the information about the full composition of fatty acids contained in the fish and soybean oils used in this study can not be provided by us, according to the information provided by the manufacturer (Naturalis®, Nutrição & Farma LTDA, Brazil) the fish oil is rich in \( \omega-3 \) since a total of 1.6g of polyunsaturated fatty acids is present in 2g of the oil, 1.1g corresponding to eicosapentaenoic acid (EPA) and 0.2g to docosahexaenoic acid (DHA). So, more than 80% of this oil is composed of \( \omega-3 \) polyunsaturated fatty acid. Regarding the soybean oil, according to the information provided by the manufacturer (Soya Distribuidora de Produtos Alimenticios LTDA), the soybean oil is rich in \( \omega-6 \) because in 12.3g of the oil there are a total of 6.7g of polyunsaturated fatty acids, since 6g correspond to linoleic acid (\( \omega-6 \)) and a minimal quantity (0.7g) corresponds to linolenic acid (\( \omega-3 \)). So, more than 89% of the total of polyunsaturated fatty acids in this oil is composed by \( \omega-6 \) polyunsaturated fatty acid. Based on these information, we can suggest that the effect of fish oil treatment can be attributed largely due to \( \omega-3 \) polyunsaturated fatty acid, whereas the results obtained in the soybean oil treatment can be attributed mostly to the \( \omega-6 \) polyunsaturated fatty acid. These pieces of information were added in the sentence related to oils description (page 9, lines 305 - 312). However, the reviewer is right, we cannot state that these effects are exclusive due to omega-3 or omega-6. In addition, we believe that it is also important to clarify that the reason to use these oils from these companies is that they are commercially available in Brazil.
Another important point to be discussed is the fact that in the in vivo experiments the cytokine/ inflammatory mediators have been measured in the tumor homogenate. Why the tumor was not excised and evaluated in toto by immunohistochemistry? This latter could allow to properly mark the presence and the localization of inflammation (mainly cytokine) into the melanoma tissue. Hence, the intratumoral or peritumoral inflammatory infiltrate should also be evaluated.

Author response: Thank you so much for these comments and suggestions. First of all, it is important to mention that in our previous reports - Bachi et al., 2009 and Bachi et al., 2012, we investigated by immunohistochemistry not only the inflammatory process, but also the immune cells infiltration (neutrophils and macrophages) into the tumor microenvironment favorable to melanoma growth in our experimental model. For sure, we would like to perform some experiments by immunohistochemistry to complementary the results showed here. Unfortunately, we could not carry out immunohistochemistry experiments due to problems to purchase the antibodies and equipments needed to do it. By the way, for sure, for further studies we intend to perform immunohistochemistry experiments.

Moreover, why the authors did not use the B16 mouse (instead of C57BL/6) as experimental model?

Author response: We would like to clarify that not only in our previous studies (Bachi et al., 2009 and Bachi et al., 2012), as well as in the study carried out by Correa et al., 2005, our group has developed experiments using different melanoma cells that were originally obtained from C57BL/6J mice. In relation to the B16F10 cells, according with the ATCC website (https://lgcstandards-atcc.org/products/all/CRL-6475.aspx), this cell is a murine melanoma cell line from a C57BL/6J mouse. So, we used the C57BL/6 mice to maintain our experimental model.

Again, since the use of fatty acids to inhibit the melanoma growth should represent a promising future treatment, the Authors might briefly discuss such point in the Discussion. Do they believe that the ω-3 and ω-6 should also be effective when administered as topical ointment?

Author response: Thank you so much for the comment and suggestion. However, although the results obtained by us in this study showed that the treatments by gavage with oils enriched with ω-3 and ω-6 (1:1 ratio) were able to reduce melanoma growth, it is very important to mention that our results were obtained in an experimental model in which the melanoma growth is dependent of an acute inflammation. So, to suggest an effective effect of the mixture of ω-3 and ω-6 (1:1 ratio) when administered as topical ointment is inappropriated now. Nevertheless, as we intend to develop further studies evaluating the effect of the use (isolated or in combination) of ω-3 and ω-6 in different ways, we can account this very good suggestion.
In my opinion the description of the results depicted in Fig 3A and 3B should be revised (lines 127-128) and the statistical significance should be stressed.

**Author response:** Thank you so much for the comment. We would like to clarify that the sentence "Twenty-one days after the coinjection, the tumors from each mice group were removed in order to obtain a tumor homogenate that was used to determine the levels of inflammatory mediators LTB4, LTB5, PGE2 and PGE3 in the tumor microenvironment." was revised and added in main text (page 4, lines 127-130). In addition, the statistical description in the legend of figure 3 was revised.

Line 155: please change higher with lower (please check it in the corresponding Fig 4D).

**Author response:** Thank you for this correction. The figure 4D was wrong and the correct figure was added in the manuscript.

Figure 5: I believe that the Graph is too much full of data, I suggest to show only the measurements obtained with 10 microliter, or maybe those with 10 and 20 μL (please remember that the mice where treated with the concentration of 10 μL). Another option is to report the other data as Supplementary Materials.

**Author response:** Thank you so much for the suggestion, but we considered that figure 5 showed important data and we intend to maintain it presentation format.

Line 206: please correct the sentence “it has was shown effectively suppress”

**Author response:** The sentence was changed to “it has shown effectively suppress” (page 6, line 208).