Author's response to reviews

Title: Growth and metastases formation of human malignant mesothelioma cell lines orthotopically implanted into the pleural cavity of immunodeficient mice.

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Author's response to reviews:

Dear Editor,

In relation to the manuscript 1398630894756530 entitled "Growth and metastases formation of human malignant mesothelioma cell lines orthotopically implanted into the pleural cavity of immunodeficient mice", by myself et al., here is a reply to the critiques of the reviewers on a point by point basis.

We thanks BMC cancer and the referees for their criticisms that significantly contributed to the improvement of the manuscript.

Reviewer 1. John Edwards

Major Compulsory Revisions:

Manuscript as been revised and spelling and grammar have been corrected.

Minor Essential Revisions:

The left hand column of table 2 represent individual animals. Table 2 legend was wrong and has been modified.

Reviewer 2. Amir Onn

Major Compulsory Revisions:

1) Injection in the pleural space was set up before the experiment with the cancer cells. About twenty nude mice were injected with colorant to determine the right site of injection in the chest and the precise profundity of the needle tip required to reach the pleural space. In the revised manuscript a statement concerning a prior setting of the technique has been added (page 3).

The cells were injected immediately under the left anterior limb, approximately in the fourth intercostals space. This information has been added in the manuscript (page3).

Image 2 is a representative image of the IST-Mes3 growth which, as explained in the discussion, grew very fast and irregularly. In some cases big tumor masses were found in the right chest, but most of the mice injected with these cells developed bigger tumors in the left chest. Picture 2 was chosen to show the irregular growth of IST-Mes3 cells. In the figure 2 legend a relative comment has been added.

No pneumothorax was evidenced and lymph node metastasis were noted only at later stages of tumor growth. Results have been updated including these informations (page 5).

2) We stated that, at present, surgery was used to implant human malignant pleural mesothelioma cells in the pleural cavity of mice and that the production of inflammatory molecules and growth factors following the surgery can interfere with the tumor growth. We do not used surgery to implant the tumors so its related
inflammatory response was avoided. No hyperemic area or exudate in the pleura were found in the site of cells injection.

3) Pleural effusion was noted only in animals with advanced tumors, but we were unable to correlate the pleural effusion with a precise tumor status. Informations concerning the pleural effusion as well as comments regarding the biphasic subtype of the cells implanted has been added in the manuscript (page 5).

Minor Essential Revisions:

1) H&D slide of mesothelioma tumor invading the lung has been presented.

2) Protocol for CD31 was reported in the submitted manuscript (Materials and Method, Immunohistochemistry). The protocol has been modified with the new antibodies used.

3) The sentence "Mouse were left to die" has been replaced with "Mice were sacrificed when moribund", as we meant to say.

Reviewer 3. Anton Berns

Major Compulsory Revisions:

In vitro phenotypic characterization of the cell lines used in this work were performed previously as reported in the bibliography (10-12). We have updated the manuscript with the analysis of mesothelioma-specific markers detected with immunohistochemistry in the orthotopic tumors (page 5).

Figure 3 was used also to show the mesothelioma tumor invading the lung. We have added a new picture to show the high vascularization of orthotopic tumors.

The speculation in the discussion that the lag phase in tumor growth relates to the requirement for vascularization has been deleted.

As for the referee 2, the sentence "Mouse were left to die" has been replaced with "Mice were sacrificed when moribund".

Several orthotopic cancer models are used to study the effect of drug treatment on cancer growth without non-invasive imaging methodology and the effects of the treatment are achieved after mice autopsy at different times. Certainly a non-invasive method would be very useful, and a consideration regarding this opportunity has been added to the manuscript (page 6).

Minor Essential Revisions:

The corrections suggested by this referee have been added and the entire manuscript has been revised for English improvement.

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