Author’s response to reviews

Title: Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology

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Author’s response to reviews:

Dear Editor of BMC Cancer,

Thank you for your time, consideration and much valuable comments to our manuscript entitled “Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology”. We have proceeded to the correction of the underlined points and suggestions and answered the highlighted questionings pointed out by the reviewers,
Please find enclosed a new version of our manuscript, which we hope will meet your criteria to be accepted for publication in BMC Cancer.

Below you will find our comments to the reviews and we are available for any further request you may need,

Best Regards,

Dr. Xavier Thuru

Comments to the manuscript "Isolation and characterization of two canine melanoma cell lines: New models for comparative oncology" for "BMC Cancer" BCAN-D-18-00269R1: Answers to “Reviewer 1”

1. Abstract/ Results: Line 55: "sample" instead of "samples" → Corrected in the text

2. Introduction/ Line 69: types → Corrected in the text

3. Material and Methods/ Cells/ Canine melanoma cell lines:

   “Here are mentioned all 4 "cell lines" and also in Fig 2 all are called "Tumor cell lines", but in the abstract and other parts of the manuscript only Ocr_OCMM1x and Ocr_OCMM2x are named cell lines and are the presented end product. Please decide if you want to mention all in the abstract, or if these are different "classes" of cell lines

   → We have detailed here the process of the end-product (“Ocr_OCMM1X & Ocr_OCMM2X) isolation process. The primary cells were not used for the study, we have just checked that there is no genetic drift between the primary culture and the xenograft derived cells after the amplification and selection in mice.
4. Throughout the manuscript there is no consistent spelling of tumour and tumor - in the text tumour and in the Fig2 tumor. - please unify

→ Corrected in the text, everything was harmonized to “Tumor”

5. Inoculation of melanoma cells/ Line 183: 2 x 10^6 cells were injected in the mice from the xenograft. How many cells were injected from the primary tumour material? Also 2x10^6?

→ Yes, the same amount of cells was injected each time. Either for the primary tumor material or the xenografted material so we can be able to compare the growth curves of both experiences.

6. Inoculation of melanoma cells/ Line 188: here the "Ocr_OCMM1X passage1" cell line should also be added to the engrafted tumour cell lines Ocr_OCMM1X and Ocr_OCMM2X, to be consistent. They also were in a mouse and were cultured after.

→ Added in the text

7. Results/ Establishment and characterization of canine melanoma cell lines Line 319: Here it is written that Ocr_OCMM2 Primary was cultured 5 month - so it's no cell line. As referred before please define the 5 products from the 2 dogs precisely.

→ We just describe here the estimation of population doubling times for the different cells (primary and xenograft derived). The first evaluations have been made 2 months after the first inoculation at day 0. All the cells have been maintained in culture for at least 12 consecutive months.

8. Results/ Line 321: please delete ocr

→ Corrected in the text

9. Results/ Figure 5A: is there any necropsy material of Nude mice from dog 2 xenografting where pictures could be added to the Figure? This would make it all more consistent.

→ Unfortunately, there are no other relevant necropsy material from Dig_2 to expose. The brains were harvested but no evidence of metastases were found. The lungs, livers and spleens showed similar invasion to the necropsy material collected for Dog_1 xenografted mice.
10. Results/ Figure 5B: Please describe the figure legend in more detail and
   → Additional information is added to text

11. Results/ Is there confusion between the cell lines? It seems that Ocr_OCMM1 is growing faster??
   → There are no confusions between the cell lines. Ocr_OCMM1 is growing faster indeed, but the curve corresponds to “passage 2”. The results for the “passage 1” are not exposed and only 3/5 mice have developed tumors and within 120 days (cf. figure 4).

12. Results/ Morphological and phenotypical characterization: Is there a possibility to add Figures of histopathology of the two primary material tumors from the canine patients, where the cell lines were made off? In general is there any photo material of the primary lesions?
   → Figures are attached separately with the answers: Additional file 3A

13. Results/ This would make the whole manuscript more consistent - in the genetic section there is the structure which would be beneficial for the whole manuscript: Tumour - Xenograft - Ocr_OCMM2Primary/ Ocr_OCMM1XPassage1 - Ocr OCMM2X/Ocr_OCMM1X. This would be beautiful also for histopathology and would really help the reader not to lose track.
   → The files have been modified accordingly and added with the additional files. The comparisons are made between Dog_2 primary tumors and Xenograft vs. cell lines of Dog_1 (Additional file 7 & Additional file 8)

Answers to “Reviewer 2”

→ None