Author's response to reviews

Title: Cytoplasmic BRMS1 expression in malignant melanoma is associated with increased disease-free survival

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Author's response to reviews:

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Editorial-in-Chief
BMC Cancer

Dear Editor

Please find enclosed a revised manuscript by Slipicevic et al. entitled "Cytoplasmic BRMS1 expression in malignant melanoma is associated with increased disease-free survival" for your consideration for publication in BMC Cancer.

We have modified the paper according to the reviewers comments and we feel that this clearly has improved the quality of the paper.

Reviewer (Samant)

1. We have included a picture (Fig 1 G, H) showing that BRMS1 is mainly localized to nucleus in the cell lines. This has also been stated in the text (page 9 and in figure legend to Fig 1)

2. We have included results (Fig 3 A) showing silencing efficiency using quantitative real-time RT-PCR. The method has been included on page 7.

3. We were asked to discuss in more detail the associations to FABP7, ERK and Akt. To do so we have included some more details in the discussion and included some new references (ref 18, 37, 35, 32).

4. We were suggested to add a pictorial summary of BRMS1 location with
respect to melanoma progression. We have not included such a picture as we do not see how this will improve the quality of the paper at this point. For instance it is difficult to in a picture describe the correlation of nuclear BRMS1 in nevi still BRMS1 localized in the nucleus is a poor prognostic factor in primary melanomas

Reviewer (Iwase)

1. We have included more pictures (Fig 1 A, G) demonstrating cytoplasmic, cytoplasmic and nuclear, and nuclear only immunoreactivity. We have in the figure legend also described the staining pattern in each figure

2. The immuno histochemical studies concerning cyclin A, Ki67, cyclin D3, p-ErK, p-Akt and p21 have been performed previously. The cutoffs were determined in these papers so that high and low expressing groups were as similar as possible and also based on what had been reported by others. References to these papers have been included also in the figure legends when appropriate

3. We have discussed with a statistician and it is not possible to analyze the data as continuous or semiquantitative score

Reviewer (Miracco)

1. The paraffin-embedded tissues used in this study have been collected from archives and mostly from patients being operated before 1992. At this time neither clinical stage nor mitotic counts were specified. We have however in ref # 22 shown in a small cohort of samples that there was a strong association between number of mitosis and expression of cyclin A. We have included this statement in the manuscript. Furthermore, based on what has been published by others regarding the association between i.e. cyclin A, Ki67 and proliferation we feel that our conclusion suggesting an association between cytoplasmic BRMS1 staining intensity and proliferation is valid.

2. We have performed an additional statistical analyses were we compared those with tumor thickness < 1 mm (radial growth phase) to those with vertical thickness # 1mm. No difference was however observed, and the findings have been described in the result section as data not shown (page 9)

3. We have included a sentence in the result section saying that BRMS1 was heterogeneously and randomly expressed throughout the tumors (page 8)

4. More pictures showing different patterns of staining have been included (Fig 1)

5. To expand the discussion regarding the contrasting results obtained in other studies we have included a paragraph were we discuss the possibility that various splice variants of BRMS1 may be expressed in a cell specific and microenvironment specific manner. Although not yet known such splice variants may have different biological function (page 14).

6. We have included mean + SD thickness
7. We have to the best of our knowledge corrected typewriting mistakes.

Hoping that the manuscript will now be accepted for publication in BMC Cancer, we look forward to receiving your decision.

Yours sincerely,

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