Author's response to reviews

Title: Spatial morphological and molecular differences within solid tumors may contribute to the failure of vascular disruptive agent treatments.

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Author's response to reviews: see over
Dear Sir/Madam,

Please find attached the revised manuscript entitled: “Spatial morphological and molecular differences within solid tumors may contribute to the failure of vascular disruptive agent treatments.” by Linh Nguyen, Theodora, Caterina Malcontenti-Wilson, Lie Sam Chan, Patricia Luiza Nunes Costa, Mehrdad Nikfarjam, Vijayaragavan Muralidharan, Christopher Christophi for consideration for publication in the Journal of BMC Cancer in the original manuscript section.

We thank the reviewers for their suggestions and constructive criticisms. We took these suggestions/criticisms into account in this revised manuscript. All changes in the revised manuscript are marked in red.

Addressing reviewers' specific points

**Referee 1:**

*The additional information in supplementary data is useful here but the authors need to ensure that inter-animal variation as well as inter-tumour variation is reflected in the plot and analysis – authors do not say whether this has been addressed. Statistical advice should be sought on how to present these data.*

We have consulted a statistician regarding the analysis of the results. Due to the number and distribution of tumour nodules it is not possible to calculate the volume of every nodule. As shown in preliminary figure 1, each sliced liver section contains 2D cross sections from many nodules and many of them, especially the larger ones, are present in a number of liver slices. We selected sections to be representative of the whole liver and often more than one section per tumor is included in the analysis. The statistician’s advice was that average value for each animal should be calculated separately and then the final result should be the mean average of all animals in the group.

*Point taken re. quantifying hypoxia. A semi-quantitative analysis of the markers shown in Fig 3 seems reasonable. However, it is incorrect to provide means and standard errors of these scores. Statistical advice should be sought on how to present these data.*
Staining was variable within different tumors and even within adjacent regions of a single tumor. Each region was scored and an average score was calculated for each animal. The mean of all average scores in each group are presented and therefore it is possible to provide means and standard errors of these scores. The statistician’s advice was that it is a legitimate way to present these results and literature supports such presentation. If the Editor however is of the opinion that standard errors should be omitted we are happy to comply.

Most of these issues have been addressed. However, it is clear from the new Suppl Fig 2 that staining for CD34 is highlighting many more tumour vessels than is apparent in the main figure 1. Better images of CD34 staining should be shown in Fig 1.

We replaced the image in Figure 1 with a new one that shows more stained vessels and highlights the lower staining in the periphery.

Also, no new high power images of a-SMA staining have been provided, rather the original high power images are no longer in the main fig 1 but appear in Suppl fig 3 (note that the title of this fig is incorrect). The results for a-SMA staining shown in Fig 1B are clear but it is still not clear how these were obtained. There is a little more information given on page 5-6 but this does not explain how vessels were identified at the periphery, where there appears to be so much staining in myofibroblasts. This needs inclusion. Was it just the very large co-opted vessels that were counted? If so, this should be made clear. Also, the number of vessels analysed for a-SMA should be given. If no better images of a-SMA can be provided, I think that the Discussion should at least outline limitations with this analysis and be somewhat more circumspect in the conclusions regarding vessel maturity in the periphery.

We selected new images for a-SMA staining that show low and high magnification of the relevant sections. High magnifications are necessary to see the vessel staining. We used serial sections to ensure the structures we identified are vessels even if CD34 staining is a lot less intense. Unfortunately this particular tumour is associated with large numbers of myofibroblasts, especially in the periphery, so it is likely that the number of vessels staining with a-SMA is underestimated. This issue has now been raised in the results and discussion.

The image for Ang 1 is an improvement. Data are still limited but as long as the a-SMA data is strengthened – see comments above – this is OK.

Additional Essential Revisions

1) All images need to be higher resolution.

We have made the images as per author instructions. However if the editor/publisher is of the opinion that the figures are not of sufficient resolution we would welcome instructions for changes.

2) Supplementary figures – title of Suppl Fig3 needs correcting. mm should be μm.

This has been corrected.

3) Page 7 – need to give the suppliers for all the antibodies.
This information is provided in the supplementary table 1.

4) Page 8 – Authors say that only viable tumour areas were analysed but in Fig 5, CD34 staining in A is clearly shown in necrotic regions and presumably included in the analysis. This needs clarifying in the text.

The statement referred to the analysis of control tumors however the reviewer is correct, it is not a valid statement for the treatment groups where while necrosis is clearly evident some vessels and endothelial cell staining are still visible. We have now left this statement out.

4) In Fig 2, the asterices showing significance on the graphs need replacing.

This has been done.

5) Page 17 – please use an original reference rather than ref 3, which is a review, for the proposal that tumor cells in the periphery survive due to close proximity to host vessels. Ref 3 is however suitable for the following statement about retained perfusion, as currently quoted.

This has been done.

**Referee 2:**

I think the authors should discuss the extent of necrosis in their tumour model or refer to their previous published work regarding this.

Appropriate references are included in the section describing this tumor model.