Reviewer’s report

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

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Reviewer: Chryso Kanthou

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In this manuscript Nguyen et al have investigated morphological and molecular characteristics of the tumour, its vasculature and stroma using a colorectal liver metastasis model and an immunohistochemical approach. The authors suggest that inherent differences between the periphery and central regions of the tumour could contribute to the failure of vascular disrupting agents to effectively target the tumour rim. The data presented in this study are very interesting but mainly correlative – there is no direct evidence provided here that the morphological and molecular differences described are directly responsible for the failure of VDAs. I would therefore suggest that the title of the manuscript is changed.

Major points

1. In page 8 of the manuscript the authors state that vessels in the tumour periphery either did not stain or only partially stained for CD34. Presumably they refer to the rather large open vessels that could be easily distinguished morphologically. However, tumour vessel morphology/size is heterogeneous and this raises the question of whether the data presented on microvascular density throughout this manuscript reflects the true extent of vascularisation of these tumours. For example, from the images presented it is not clear whether the antibody picked up smaller vessels at all? The authors should consider testing another endothelial marker such as CD31 in their system. Also the authors should describe how they quantified MVD and pericyte coverage in more detail and explain what the numbers in the bar charts presented in Figure 1 mean? Also, how were pericytes distinguished from myofibroblasts if not all blood vessels could be detected.

2. Figure 3: the central region of the tumour in the top left panel looks quite normoxic? In addition, there are rim areas within the same section (to the right) that look quite hypoxic? The authors should provide quantification of the hypoxic and normoxic tumour area in relation to spatial distribution.

3. Figure 3: how representative is the VEGF peripheral staining shown in the middle row of this Figure? Did all tumours stain similarly?

4. Figure 3, bFGF staining: where does the tumour tissue end and liver parenchyma begin? Parallel H&E stained sections would be useful here.

5. Figures 5 and 6 caspase staining: if vessels in the central regions failed to stain with CD34, then how did the authors localize and quantify caspase activity in the vasculature of treated tumours? How were caspase-positive tumour...
vessels defined and how was the distinction made between vascular endothelial and tumour cell apoptosis? What was the level of apoptosis in control untreated tumours?

6. Page 11. The authors state that the vessels became “leaky” after Oxi4503 but have not assessed this with any functional assays.

Minor points

7. The authors state how many tumours they analysed per treatment group but do not say how many animals per group these represent. This information is needed for each of the analyses presented.

8. All figures need appropriate scale bars

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

No competing interests