Reviewer's report

Title: VEGFR2 heterogeneity and response to anti-angiogenic low dose metronomic cyclophosphamide treatment: role of vascular normalization

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Reviewer: Darrell Yamashiro

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This manuscript by Patten et al, examines the effect of heterogeneous VEGFR2 expression in response to low dose metronomic cyclophosphamide (CTX) anti-angiogenic treatment. They examine this in xenograft models of both malignane melanoma and colorectal carcinoma. By studying the microvascular density, tumor hypoxia and blood vessel normalization, they find that heterogenous expression of VEGFR2 may not affect response to low dose metronomic cyclophosphamide treatment. Although the area of study is interesting, results presented in this manuscript are variable, and no positive conclusion can be drawn.

Major Compulsory Revisions

1. In Figure 1/table 1 there is quantification of VEGFR2 and CD31 staining. From Methods it is unclear how a vessel is defined. The CD31 staining is often discontinuous in a vessel, e.g. Fig 1E, near right *, is this counted as 1 vessel or separate vessels? The CD31 staining is also problematic in that autofluorescent RBCs are seen Fig 1E,F on left upper edge of images. Is this corrected by the imaging program? Also, in Methods for xenograft tumors, 5 random fields were picked per tumor, which for SW480 (4 tumors, table 1) is only 20 images to quantify. Lastly, what does VEGFR2 positive staining mean when there is no CD31 staining as there are many VEGFR2+ objects? With these issues it is difficult to interpret their results.

2. In Figure 4, again the definition of a vessel is pertinent. Fig 4A there is a large “Y” shaped vessel, is this 1 or many vessels? For E-H, the lack of correlation of the Western blots for VEGFR2 with the change in VEGFR2+ vessels is puzzling. They comment that WM239 cells also express VEGFR2 (p13), if true this makes the Western blot uninformative. One could try species specific primers for VEGFR2 and do quantitative PCR.

3. In Figure 5, HIF1a expression is measured. Since VEGF is a downstream target of HIF1a this should be determined. This information on VEGF expression (both between cell lines and with CTX) is critical also for their discussion on VEGFR2 and the potential difference between melanoma and CRC

Minor Essential Revisions

4. Figure 6C. The lower gel of CD31 expression in SW480 is too messy to get any meaningful quantification.
Discretionary Revisions

5. In Figure 3A and 3B the MVD is decreased in response to CTX. What marker is used to determine MVD (?CD31). In C & D TSP-1 expression is examined. TSP-1 data would probably be better in a different Figure or in supplemental data, as 3A and 3B is related to Figure 4.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests