Reviewer’s report

Title: Establishment of Hewga-clear cell sarcoma, a new clear cell sarcoma cell line, and investigation of the antitumor effects of pazopanib on Hewga-CCS: an in vitro and in vivo study

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Reviewer: Ryan Roberts

Reviewer’s report:

In the current manuscript, the authors present the generation of a novel fusion gene-positive clear cell sarcoma and show data suggesting both potential for treatment with pazopanib (an agent that has been suggested to be of some utility in this family of tumors before) and show some data for the likely mechanism by which pazopanib, which is a multi-targeted agent) exerts its effect. The paper is generally well-written and data is presented in a way that is very understandable. The observations are likely to be of interest for physicians caring for patients with this very rare disease and to researchers studying both multi-targeted RTKi’s and clear cell sarcoma. There are, however, a number of questions raised by some decisions the authors have made that need to be addressed to make this paper acceptable for publication:

MAJOR COMPULSORY REVISIONS:

1. Generalizability and innovation. Probably the biggest weakness of this paper is a lack of data that would suggest that these findings could be generalized to other tumors, even to other people with CCS. This could easily be done by including other cell lines in both the in vitro and in vivo experiments. While the number of appropriate cell lines is small, even experiments with one or two other cell lines, especially including those which have Type I fusion genes would go a long way to making the generalizability of these finding more obvious. Alternatively/additionally, it would have been helpful to see why the investigators chose pazopanib specifically. Were there other agents that were less effective? Additionally, it would have been nice to see whether there was an improved response with combined therapies, such as those that have been used/suggested in clinical studies. While all of these things may be a lot to ask for a journal like BMC Cancer, some effort to generalize would be helpful.

2. Clinical course of patient. Since the paper suggests that the researchers jumped straight to evaluation of pazopanib for this tumor, it is interesting that there is no mention of the regimen with which the woman from whom this cell line was derived was treated. Specifically, there is no mention as to whether she received pazopanib. It would be important information to know if she had any kind of response to the agent. It would have been reasonable to think that someone with her clinical scenario would have been exposed to the drug.
3. Lack of survival studies. The authors show delay in growth for a short timeline with pazopanib. It would be generally accepted to also show survival benefit, and to extend the growth observations beyond 28 days. How long does the growth benefit last? This data would be very important to making inferences for clinical therapies. Ideally, the authors would show both growth inhibition and survival curves.

4. In Figure 4C, there appear to be either problems or important unexplained phenomena. The p-MET timeline and p-Erk/AKT timelines don't match and show irregularities. There should be more than one sample per timepoint. IF the patterns hold for multiple samples as shown in the figure, there should be some effort to suggest why p-MET activity is higher at 24 hours than at time 0, why pErk is going down when p-MET is going up, what happened to p-AKT at 6 hours, and why this matches so poorly with the in vivo data. This might be explained, at least partially, by reference to published literature, but no attempt is made. Again, this data would also be much stronger by limiting the number of timepoints and expanding the number of tumor lines.

MINOR ESSENTIAL REVISIONS:

5. In Figure 4a, the dot blot is grossly underexposed. Given this, it is difficult to justify the inferences made. Also, it would be nice, with appropriate exposures, to see treatment/non-treatment dot blots to see what pathways have changed.

6. Since the claim is made that the tumor recapitulates morphologically the primary disease in the patient, the patient's micrographs should be shown together with those from the xenograft model in Figure 2.

7. Statistical testing for Figure 6 should utilize some type of repeated measures analysis--data are not independent.

DISCRETIONARY REVISIONS:

8. For the amount of work shown in the paper, the author list is awfully long. This may be appropriate, though the reviewer would ask the authors to ensure compliance with internationally-accepted standards for authorship.

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.