Reviewer's report

Title: In vivo dual targeting of the oncogene KCNH1 by calcitriol and astemizole results in enhanced antineoplastic effects in breast tumors

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Reviewer: Prem Sinha

Reviewer's report:

In the manuscript “In vivo dual targeting of the oncogene KCNH1 by calcitriol and astemizole results in enhanced antineoplastic effects in breast tumors” authors have probed for the antineoplastic effects of calcitriol and astemizole in mice xenografted with two different human breast cancer cell lines. The authors illustrate that the combined treatment of these two drugs results in the downregulation of EAG1 expression in the tumor tissue. Finally, the authors show that the concomitant administration of these drugs inhibits the tumor growth more efficiently than each drug alone.

The work is of interest and it will be really ideal if the EAG1 double blocking strategy turns out to be useful for patients bearing EAG1 and VDR-positive solid or metastatic tumors. The experiments are in general scientifically sound. There are some important points that should be addressed before final publication of this manuscript in BMC Cancer.

Minor Essential Revisions:

1. The mRNA level of Eag1 in figure 4 for T-47D cell line and protein level in Figure 5 does not agree with each other in astemizole treated tumors. Furthermore, the quantification of 130 kDa (Figure 5B) and 110 kDa (Figure 5C) EAG1 levels does not seem to match with the top panel (Figure 5A). This needs to be checked and clarified accordingly in the “Results” section.

2. In a previous work, this group has shown the up-regulation of VDR, while balancing the calcitriol mediated induction of CYP24A1 by combitorial treatment in breast cancer cell lines. It would be informative to see levels of these proteins (VDR and CYP24A1) in the xenograft tumors.

3. BMC Cancer does not encourage the “data not shown” policy. Further, it will be great for the readers if the authors include the data in the manuscript describing the “effect of the two drugs alone or in combination modifies EAG1 gene expression in brain tissue that normally expresses EAG1”.

4. The title of the paper contains KCNH1, while the word KCNH1 has not been used anywhere else in the entire manuscript. Likewise, the key words KCNH1 and Kv10.1 are not used throughout the manuscript.

5. Discussion section, Line 11: the sentence “Taken together these observations…..blocking of EAG1” is not very clear.
Additional suggestions-

1. It is proposed that the anti-tumor effect of the combined treatment is coming from down-regulation of Eag1. It would be informative to carry out the rescue experiment by over-expressing Eag1, to show the extent of Eag1 participation in this phenotype.

**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:**

I declare that I have no competing interests.