Author's response to reviews

Title: Axitinib and crizotinib combination therapy inhibits bone loss in a mouse model of castration resistant prostate cancer

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Author's response to reviews: see over
Axitinib and crizotinib combination therapy inhibits bone loss in a mouse model of castration resistant prostate cancer

Dear Editor,

We thank you and the reviewers for very constructive suggestions and a positive feedback. We have addressed all the concerns of reviewers within the scope of the manuscript. The corrections are highlighted in “red” in the revised version. Please let us know if you have any questions.

We look forward to hear the acceptance of the manuscript for publication.

Thanks,
Anand Giddabasappa

Authors Comments to Reviewers:

Reviewer: Colin Rae

Discretionary Revisions:
1. Due to the stated importance of angiogenesis in disease progression and metastasis, the study may be enhanced by a demonstration of the effect of the drugs (alone and in combination) on blood vessel formation or endothelial cells proliferation.

We understand that angiogenesis is an important mechanism that regulates tumor progression and metastasis. We did not evaluate anti-angiogenic mechanism in this study/manuscript, as we (our colleagues) have already shown the Crizotinib (PF-231066) and Axitinib (AG-013736) shows anti-tumor and anti-angiogenic effects by inhibiting c-MET and VEGF pathways, respectively (Zou et al., 2007 and Hu-Lowe et al, 2008). In the previous version of the manuscript, we had referenced only Hu-Lowe et al., manuscript. In the revised version, we added the anti-angiogenic effects of Crizotinib and referenced the Zou et al., article in the discussion section. Please refer the discussion section of revised manuscript (Page 13, line 10-12).

2. A comparison of the drug combination used in this study with cabozantinib would have been useful, although may be beyond the scope of this manuscript.

Yes, we agree the reviewer. Although it will be very interesting to evaluate in parallel with cabozantinib, it is out of scope of this manuscript.

3. Although the growth of tumors in castrated mice was slower than in intact mice, tumor sizes were still suitable for the experiments (and were similar according to BLI measurement in Fig 3). However, PSA levels were “below level of detection” in castrated mice, suggesting that PSA levels do not correlate to tumor size and are not a suitable marker. The usefulness of PSA or alternative markers could be discussed.
Yes, we agree with the reviewer that PSA is an unreliable biomarker for PCa. In the revised manuscript, we have added a sentence in results section (Page 11, line 1-2; thus suggest PSA may not predict the tumor burden.) to address this concern.

4. It is stated repeatedly that the combination treatment is effective. However, in both intact (Fig 3A) and castrated (Fig 3C) mice, administration of the drug combination had no greater effect than axitinib alone. This should be addressed in the Discussion.

Thanks for the comments. We have addressed the suggestion in the discussion section with below statements (Page 13; Line 26-30).

“Our data showed that axitinib alone or axitinib and crizotinib in combination reduced the tumor burden in both intact (androgen positive) and castrated (androgen negative) mice models (Figure 3A and 3C). Interestingly only the axitinib and crizotinib combination showed improvements of bone volume (BV/TV ratio) in both models (Figure 4B and 5B).”

Reviewer: Joanne Edwards

However I think in order to enhance the manuscript I suggest the following as discretionary revisions

1. The authors should display the PSA levels in the mouse following castration, this in combination with the tumour growth would conclusively demonstrate that the tumours were castrate resistant.

   Since the PSA levels are below detection level of the kit, we don’t think it adds any value to include a graph. However, we have discussed this in the results section (Page 11 – Line 1-2)

2. IHC images of the AR in addition to the western blots as cellular location of the receptor is also important.

   We agree with the reviewer that cellular location of AR is important as shown by many research groups. We did not do the experiment to evaluate the cellular localization for two reasons: (1) It is shown that PCa cell lines become constitutively active following mutations of ligand binding domain of AR and thus may cause blockade. Charles Saywer’s group has demonstrated this phenomenon in VCaP cell line and thus we did not intend to repeat this experiment. (2) Our results showed that VCaP tumor growth was independent of androgen stimulation and thus the AR localization though may be interesting, is not be necessary for this study.