Reviewer’s report

Title: Dual regulation of cell death by Akt kinase inhibitor MK-2206 in colorectal cancer

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Reviewer: Sankar Sanyal

Reviewer’s report:

Evaluation report of the paper entitled ‘Dual regulation of cell death by AKT kinase inhibitor MK-2206 in colorectal cancer

Author – Ekta Agarwal et al.

Institute – Eppley Cancer Centre, University of Nebroska Medical Centre, Omaha, Nebroska, USA.

Recommendation – Should be accepted with minor revision.

General Comments:
PI3K/AKT or protein kinase B associated cell survival signalling is now established as a promising molecular target in many cancers and therefore any drug developed to counter its effect in cell killings and the molecular insight in apoptotic cell death is welcome. It reports Akt inactivation by MK2206 in induction of mitochondria to nuclear localization of AIF in caspase – independent cell death and also Ezrin de-phosphorylation at T567, thus leading to disruption of Akt –p Ezrin XIAP cell survival complex. The paper describes dual inhibition of the TGF#/PKA/PP2A mediated tumor suppressing signalling for Akt phosphorylation and also dissociation of the survivin/ XIAP complex . The paper poses relevant questions, offers clear objectives, adequate methodologies and meaningful discussions of the results, and therefore in my opinion should be published in BMC cancer. However, the authors should also address the minor issues as listed below:

1. Title: “Dual regulation of cell death” can be suitably expanded to allow a glimpse of the content of the paper to the prospective reader.

2. Corresponding author: Is it a policy of the journal to mention the ‘co-corresponding author’.

3. Reference 4 ‘Cell Signal’ is incomplete.

Methodology:

1. DNA fragmentation assay: The classical ladder formation could have been done and shown as a supplementary data.

2. Western blot: Electrophoretic transfer must have been checked on a
nitrocellulose membrane by Ponceau S, and that may be mentioned.

3. Control: In cell culture experiments, the control must have received the vehicle of the drug DMSO for MK-2206.

4. Make for the Confocal microscope must be given.

5. Xenograft studies: How the dose level of MK-2206 at 120mg/kg body weight had been arrived at? Previous references should be given. Also the duration of 3 weeks in alternate days should be clarified.

6. Animal number: What does it mean by ‘at least 3 independent control....’
   Mention the exact number of animals and repetition to validate the results. Animal pictures, before and after treatment of MK-2206, at least at the final stage could be shown as a supplementary figure and legend.

7. Tumor microimaging system should be a little bit explained and properly referenced.

8. How many cells were counted for TUNEL assay?

9. Fig 2B: Is the cell death with MK-2206 strictly in a concentration dependent manner.

10. Page 12: Bcl-xl caption for Fig 2E is missing in the figure (IP for Bcl-xl).

11. It should be ‘g’ (tumor wt) and not ‘gms’ as written in the text and fig 3D.

12. Fig S4 - Was the densitometric data of tAkt normalized with GAPDH.

13. Legend fig 3B: Reduction.......after different days of drug treatment etc.

14. Legend fig 6B and C have been interchanged, please check.

15. Fig 7 C: Western blot of XIAP had not been shown although mentioned in the legends.

16. Fig 6 B: Is it really a confocal image, that too taken at 60 X or is it a simple flouroscence microscope, please clarify. Also caption B and C have been interchanged in the legend.

Discussion:

Specificity: My only problem with MK 2206 is its specificity on PI-3K/Akt signalling. Has anyone checked the molecular cross-talks with this compound with other targets such as the oncogenic protein COX-2, redox sensitive transcription factor NF-#B, or the pleckstrin homology domain containing protein such as PTEN or tyrosine kinases belonging to Btk/ltk/Tec family of proteins. A molecular cross-talk between the different pathways cannot be ruled out.

**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.