Reviewer's report

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

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**Reviewer:** Manfred Jücker

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In this manuscript by Agarwal et al., the authors describe the effects of the AKT inhibitor MK-2206 on human IGF1 receptor-dependent colorectal cancer cells in vitro and in a xenograft mouse model in vivo. The authors characterized the molecular mechanisms by which MK-2206 induced cell death and conclude from their data i) that AKT inhibition is effective in reducing tumor growth of IGF1R-dependent colon cancer cells in the xenograft mouse model ii) that the observed effects by MK-2206 is mediated by the upregulation and nuclear localization of AIF in a caspase-independent manner and by inhibition of the AKT-Ezrin-XIAP signalling axis in a caspase-dependent manner due to inhibition of AKT2 and iii) that these results provided a preclinical rational for therapeutic targeting of the subset of IGF1R-dependent cancers in CRC.

The manuscript is well written and the identification of two novel mechanisms by which MK-2206 mediates cell death in a caspase-dependent and caspase-independent manner is very interesting. However, there are a few points which have to be further addressed.

**Major Compulsory Revisions**

The authors claim that MK-2206 inhibits the expression of XIAP, however this is not clearly shown in Fig. 2c. A quantification of this experiment has to be shown. In addition, down-regulation of XIAP has to be shown in the xenografts after MK-2206 treatment (Fig. S1).

The reduced binding of phosphoBad to 14-3-3 shown by co-immunoprecipitation is due to the reduced phosphorylation of BAD (S136) as shown in figure 2c. The immunoblot has to be performed with an anti-BAD antibody instead of an anti-phospho-BAD antibody to show a reduced binding of BAD to 14-3-3.

The implication of AIF in the MK-2206-mediated effect on caspase-independent cell death is shown by an AIF inhibitor at a high concentration of 50 µM/L. This inhibitor may not be specific anymore at this high concentration. To proof the implication of AIF in the MK-2206-mediated cell death, the authors should down regulate AIF by siRNA and measure the change in cell death after MK-2206 treatment in comparison to an MK-2206-treated scrambled control.

The authors report in the results (page 15) that knock down of Ezrin resulted in complete loss of XIAP and survivin (data not shown). These data are important to
explain the proposed mechanism and have to be shown in the manuscript.

Discretionary Revisions

The figure legends should describe the experiments performed only, but should not contain the conclusion made by the authors e.g. Fig 2E..."The interaction increases on treatment with MK-2206 thus leading to increased cell death". This interpretation belongs in the discussion and has to be removed from the figure legend.

The authors have analyzed AKT1 and AKT2, but not AKT3. It would be interesting at least to investigate the expression of AKT3 in the colon cancer cell lines, analyzed. If AKT3 is expressed, it would be interesting if down regulation of AKT3 has the same effect as observed after down regulation of AKT2.

Minor Essential Revisions

Page 28, figure legend 6C: …in treated as compared to control cells.

There a few typing errors, e.g.
Page 7, at the end of the page: immuoprecipitation (immunoprecipitation)
Page 8, first line, second paragraph: Akt21si (should be Akt1si).

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'