Reviewer's report

**Title:** Synergistic growth inhibition by acyclic retinoid and phosphatidylinositol 3-kinase inhibitor in human hepatoma cells

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**Reviewer:** Carmen Blanco-Aparicio

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Baba at al. claim that inhibition of RXR# and PI3K in hepatocarcinoma cell lines is synergistic in their antiproliferative activity inducing a higher level apoptosis and a stronger downregulation of both pathways. The authors clearly demonstrate the synergism and a possible molecular mechanism, by the combination of ACR and LY294002 in different hepatocellular carcinoma cell lines.

**Minor compulsory Revision:**

The authors show that ACR and LY294002 alone have a moderate antiproliferative activity in hepatocarcinoma cells lines, but do not have any effect in Hc normal hepatocytes. Later they show that the combination is synergistic in hepatocarcinoma cell lines, but what happens with Hc normal hepatocytes do they render insensitive to the combination?

In figure 2A which is the effect of LY294002 or ACR at the single dose tested alone, this should be represented in the graph to visualize that the combination has a higher effect than each agent alone.

Authors said that data obtained in figure 2A was used to calculate the CI. From which graph 2A was done. The graph in which ACR is in dose response seems to be more appropriate to do so, as the effects of the different doses of LY294002 are much clear.

Have the authors tried to repeat the experiments with a more potent PI3K inhibitors available in the market, to see if stronger effects are obtained.

Is the combination effect dependent on the status of the PTEN/PI3k/AKT pathway of the hepatocarcinoma cell lines. How Do the cells that have the pathway activated respond in comparison with the cells that do not have the pathway activated?

The authors treat the cells for 12h to see an effect on the phosphorylation of different proteins. Why they do a so long time, usually inhibition of a phosphorylation is a very fast process. Do the authors have done a time course for the downregulation of the biomarkers?

**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.