Reviewer's report

**Title:** Panobinostat synergizes with bortezomib to induce endoplasmic reticulum stress and ubiquitinated protein accumulation in renal cancer cells

**Version:** 2  
**Date:** 7 May 2014

**Reviewer:** Dae Kim

**Reviewer's report:**

This article by Sato et al explores the HDAC inhibitor, panabinostat, and by combining bortezomib, the degradation of these proteins are inhibited, facilitating ubiquitination and oxidative stress. I do like the idea of using both of these mechanisms to induce oxidative stress. However, please address the following suggestions before the manuscript is acceptable for publication.

**Major compulsory revisions**

1. Please perform proper controls. In figure 2, when panobinostat and also bortezomib was not used (0 nm). Were experiments performed with DMSO to ensure the proper controls were performed.

2. Synergy describes the interaction of two entities to produce something that is greater than the sum while it is unclear from the data presented if the result is rather additive as figure 5 demonstrates induced ER stress and histone acetylation. I would suggest RT-PCR of the aforementioned select proteins to truly demonstrate synergy.

**Minor Essential revisions**

1. Please explain why the aforementioned concentrations of 50 nM of panobinostat and 10nM of bortezomib were used as the cell line Caki-1 demonstrated combination index <1 for 25 nM and 10nM for panobinostat and bortezomib, respectively

2. In figure 2B, the colony formation assay demonstrates % number of colonies to be drastically different for combination treatment. When the cells were treated for 48 hrs, why was it necessary to wait 1-2 weeks for incubation? Why not a definite time point of 1 or 2 weeks.

**Discretionary Revisions**

1. The authors may comment on the mechanistic role of panobinostat and bortezomib on affected pathways as it has been suggested in lymphoma. For example, caspase pathway, stat 5 and stat 6 phosphorylation, and the hif1 pathway.

**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:

None