Reviewer's report

Title: Enhancement of hypoxia-activated prodrug TH-302 anti-tumor activity by Chk1 inhibition

Version: 2 Date: 10 February 2015

Reviewer: Ester Hammond

Reviewer's report:

It is not clear to me if this is a revised manuscript or a first submission. I do not recall reviewing it previously.

Over all the manuscript provides a detailed and interesting report of the potential for combining the HAP TH302 with Chk1 inhibitors. I recommend the manuscript be accepted with few (text) changes.

The introduction provides an up to date, concise description of both TH302 (the leading HAP in the field/cinal trail) and Chk1 inhibitors. However the combination of DNA damaging agent and Chk1 inhibition is far from novel. Indeed all the Chk1 clinical trials run to date have been in combination with a DNA damaging agent. There are also examples of Chk1 inhibitors modified for use in hypoxic conditions, form Sentinel Oncology and ACS Chem Biol. 2013 Jul 19;8(7):1451-9. doi: 10.1021/cb4001537, both should be referenced (although I realise that might be difficult for the Sentinel compound).

The efficacy of Chk1 i activity in p53 null vs wild type cells is also not novel but nicely demonstrated here.

The hypoxic conditions need further clarification. What does N2 mean? were the cells not provided with carbon dioxide?

HT29 cells are not described until line 357, please move this to where they are first used.

I am surprised that the Chk1 inhibitors showed no toxicity alone and even more so that no increase in tail moment was seen. Some breaks would be expected during normal replication in the absence of Chk1, however it is likely that because this would be at a low level and only in S phase cells the comet assay may not have detected them. Perhaps acknowledge that a low level of damage would be expected.

Overall, a nice study clearly demonstrating the effectiveness of TH302 in preclinical models.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests