Reviewer's report

Title: Combined inhibition of the cell cycle related proteins Wee1 and Chk1/2 induces synergistic anti-cancer effect in melanoma

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Reviewer: Raghavendra Gowda

Reviewer's report:

Acquired resistance provides a formidable obstacle to single drug targeting therapy for metastatic melanoma. Combinatorial treatment along with efficient delivery of reagents augments tumor inhibition by synergistically impeding “driver” oncogenic pathways and locally increasing drug dosage and availability. In this manuscript, Gry Irene et al. reported that combinatorial treatment with a WEE1 inhibitor and a Chk1/2 inhibitor synergistically restrains the growth of metastatic melanomas. Compared to either inhibitors used as single agents, combined treatment decreased spheroid growth and additionally benefit tumor inhibition in vivo. This study has potential importance, but there are some serious conceptual and technical limitations, which prevent the assertion of the main conclusions.

1. Instead of dose curve, please provide the IC50 values of MK1775 and AZD7762 inhibitors with all the respective cell lines.

2. The calculation of combination index (CI) in Table 1 is seems to be inaccurate in the manuscript. As described, CI values are not matched with the Figure 1C. In all most all the cases, combined treatment inhibition is very close to alone treatment. These issues might potentially limit the translational significance of the study.

3. In Figure C, the doses are not mentioned clearly either in Methods or legend sections. In addition, most experimental methodology and legend sections information is also not readers friendly, please revised these sections seriously.

4. In Figure 3, the combination effect in tumor size is very weak and most importantly the toxicity in animal groups those who received MKK1777+AZD7762 was not discussed.

5. In Figure 3, I certainly confused the administration route that authors used in these studies. For example, Control DMSO used for DMSO (route not mentioned anywhere), MK1775 used in oral ?, AZD7762 used intravenously ? the combination of drugs route is not mentioned ? This is really hard to understand how the experiments was conducted by authors, please provide the reason of various drug administration route used and how they will be comparing the effect of drug and the bioavailability ?

6. In Figure, the Western blot bands almost burned OUT, it is difficult to assess
the results; author should provide the lighter exposure. In addition, γ-H2A.X did not see any combinational effect in WM45.1 and WM983B cell lines?

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.