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: Manmeet Raval

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This is a clearly written manuscript investigating the synergistic effects of combined inhibition of Wee1 and Chk1/2 kinases in metastatic melanoma cells. The stronger effect of combined inhibition as compared to mono-inhibition is of particular interest as it holds promising aspects to further evaluate the use of Wee1 and Chk1/2 as a potent targets. The results demonstrate that the combined inhibition of Wee1 and Chk1/2 in various melanoma cell lines leads to a stronger inhibition of cell viability in monolayer cultures and 3D-cultures, and irreversible inhibition of growth in 3D-cultures as compared to mono-inhibition. Combination index (CI) values of combined treatment with Wee1 and Chk1/2 inhibitors demonstrated cytotoxic synergy. Subsequently the authors show that the combined inhibition of Wee1 and Chk1/2 leads to significant reduction in tumor growth of the melanoma xenografts as compared to the mono-inhibition, these results well complements the in vitro data. They then show the effect of MK1775 and AZD7762 on the CDK1 phosphorylation, and DNA damage and apoptosis markers in the treated melanoma cells. Increased expression of #H2A.X and increased cleavage of Caspase 3 and PARP upon combined inhibition of Wee1 and Chk1/2 is indicative of DNA-damage and apoptosis. However, the combinational siRNA knockdown of Wee1 and Chk1/2 had less effect on DNA-damage and apoptosis markers. Then they narrow gated the cells in different phases of the cell cycle based on phosphoHistone H3 (mitotic marker) and treated them with MK1775 and AZD7762 mono- or combined- therapy and showed that the combined inhibition of Wee1 and Chk1/2 led to increased fraction of cells undergoing premature mitosis. They also examined the #H2A.X median values of the narrow gated cells treated with Wee1 and Chk1/2 mono- or combined inhibition and found that the majority of S-phase cells had increased #H2A.X expression which is indicative of DNA damage.

Although the manuscript is relatively brief and some of the in vitro assay demonstrating combinational inhibitory effect of Wee1 and Chk1/2 on cancer cells has been previously reported (Russell et al., 2013 and Guertin et al., 2012), nevertheless, this study focusing on its effect in metastatic melanoma cells is a significant result.

Other than the relatively minor essential revisions listed below, the manuscript is clear and appropriate, and the conclusions are justified.

1) Figure 1, 2 & 4: For all the quantitative assay results although the decrease in cell viability with MK1775 and AZD7762 combinational therapy is apparent and
the error bars (standard deviation) appear correct, no statistical significance (p value) has been provided. Specifically, p-value for the differences among combined- and mono-inhibition in FEMX-1 in figure 1C and for WM239 cells in figure 2C. It should be specified in the text if the differences are not statistically significant.

2) Figure 1C: It is intriguing that Chk1/2 inhibition in WM983B cells yielded similar decrease in cell viability as combined Wee1-Chk1/2 inhibition. Does it show a similar result when treated with a lower dose of AZD7762? Assuming that there was no other variable, could there be a possibility of potential interplay between Wee1-Chk1/2, such that inhibition of Chk1/2 leads to inhibition of Wee1? This is a minor point but perhaps this result could be more explicitly stated.

3) Line 61: The relevant reference for the survival period data should be specified. The indicated survival period is for patients of what age and gender?

4) line 226: “exception to this was WM983B cells were single-agent treatment with..” should be corrected to “exception to this was WM983B cells, where single-agent treatment with..”

5) line 256: “drug-combination of the inhibitors contra those in the control…” should be corrected to “drug-combination of the inhibitors in contrast to those in the control…”

**Level of interest:** An article whose findings are important to those with closely related research interests

**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.