Reviewer's report

Title: WEE1 inhibition sensitizes Osteosarcoma to Radiotherapy

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Reviewer: Marie Fernet

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Title: WEE1 inhibition by PD0166285 sensitizes osteosarcoma cells to irradiation-induced cell death

The manuscript describes the cellular response of three osteosarcoma cell lines to irradiation combined with a Wee1 chemical inhibitor. The results show that the G2 checkpoint is abrogated by the combined treatment, suggesting that this compound could be promising to enhance radiotherapy effect in patients.

Overall this is an interesting and well-written manuscript but data presented are not enough sound to conclude about the mechanisms involved in the radiosensitization by Wee1 inhibition. The efficacy of this molecule on the G2/M checkpoint was already described in several other tumour models and the only new information given by this work is its efficacy on osteosarcoma cells but molecular demonstration is missing.

Here are some suggestions to improve the quality of the manuscript:

Major Compulsory Revisions

1. The background section gives a good overview of the osteosarcoma treatment modalities. The paragraph on the G2 checkpoint mechanism does not need to be so detailed as it is well known and a figure is presented. The background section would benefit from adding additional information on use of Wee1 inhibitors in preclinical/clinical studies, the recent review by Stahis & Oza (Drug News Perspect. Sept 2010) have to be added. In addition, the statement of the question posed by the authors is missing at the end of the section.

2. The methods section would benefit from adding the dose-rate used for irradiation. Why the dose of 20 Gy is cited in two paragraphs but does not appear in results figures?

The origin of the tissue samples needs to be specified in the methods paragraph.

The radiation dose used for Western Blot analysis has to be specified in methods or in the figure legend.

3. Overexpression of Wee1 protein was described previously for several types of cancer (see Shahryar et al. Cancer Cell, 2010). The overexpression of this protein in osteosarcoma tissues is a prerequisite for the use of the inhibitor on this model, it is well presented in figure 1. For the rest of the data, cell lines were used, it should be useful to present Wee1 protein expression in these cell lines.
compared to normal cell lines by western blot in the first figure. The western blot presented in figure 2 can be moved to the first figure, with normal cell lines added.

4. The effect of Wee1 inhibition on cell survival is convincing, even if it is hard to interpret the end of the survival curves. The parameter used to make statistical analysis given in the figure legend must be indicated.

5. The description of the effect of the inhibitor on cell cycle progression is clear but the results are over interpreted. Indeed, data show that the G2 arrest is abrogated by the combined treatment but the authors did not characterized the cell death and did not investigate the DNA damage in the different conditions so they cannot conclude that the effect observed on cell survival is due to progression into mitosis with DNA damage that leads to cell death.

If the hypothesis of the authors is correct the cells should die due to mitotic catastrophe. To demonstrate this statement, the minimum experiments to do are : (1) to make a labelling of mitotic cells (with phospho-histone H3 for example) to show by flow cytometry an accumulation in the M phase, the PI analysis presented indicating only the presence or the absence of an accumulation in G2 and/or M phase. (2) To show the presence of an excess of cell death in the presence of the inhibitor. Some evidence of cell death by mitotic catastrophe has to be given. A good way to demonstrate an excess of mitotic abnormalities could be the quantification of supernumerary centrosomes by immunofluorescence.

6. The limitations of this work are not well stated in the discussion. These data are too preliminary to conclude about this Wee1 inhibitor potential use in clinic. More details have to be added to explain future experiments that have to be before clinical use of this compound.

**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.