Author's response to reviews

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

Authors:

- Gry I Magnussen (gry.magnussen@rr-research.no)
- Elisabeth Emilsen (eee@ous-hf.no)
- Karianne G Fleten (Karianne.Giller.Fleten@rr-research.no)
- Birgit Engesæter (Birgit.Engesater@rr-research.no)
- Viola Nähse-Kumpf (Viola.Naehse@rr-research.no)
- Roar Fjær (Roar.Fjar@medisin.uio.no)
- Ana Slipicevic (Ana.Slipicevic@rr-research.no)
- Vivi A Flørenes (vivi.ann.florenes@radiumhospitalet.no)

Version: 3 Date: 15 April 2015

Author's response to reviews: see over
Dear Editor

Please find enclosed a revised manuscript by Magnussen et al. entitled "Combined inhibition of the cell cycle related proteins We1 and Chk1/2 induces synergistic anti-cancer effect in melanoma"

We have modified the paper according to the reviewer’s comments and we feel that this clearly has improved the quality of the paper.

Reviewer Raghavendra Gowda

1. We were asked to provide a Table with IC50 values to demonstrate the effect of MK1775 and AZD7762 instead of Figure 1 A and 1B. Only two (WM983B and WM45.1) of the six cell lines reach IC50 values with inhibitor concentrations up to 1 uM. We think that further increase in the concentrations will result in many off-target effects and make conclusions based on these observations unreliable. For this reason we feel that a figure visualizes the effect of the inhibitors in a more detailed manner than only IC50 values. It should, however, be pointed out that the IC50 values for WM983B and WM45.1 cells were provided in the result section of the manuscript. However, if the editor still wants us to remove Figure 1 A and B we will certainly do so.

2. The reviewer claims that the combination index (C.I.) values in Table 1 are inaccurate and not matched with Figure 1C. As pointed out in the text the C.I. values are based on the average results provided in Supplementary Figure 2 and not Figure 1C. Since C.I values are calculated based on the Chou-Talalay method they cannot be directly compared to the histograms in Figure 1C. In order to highlight this better we have now included a footnote to Table 1.

3. As stated by the reviewer “In Figure C, the doses are not mentioned clearly….”. We are not certain to which figure the reviewer refers to. We have therefore to our best ability revised all figure legends to included drug concentrations and exposure time where this seems to absent. We have also referred to part of the Material and method section and Figure legends to make it more reader friendly as suggested. We noted that in Figure 4 B-E, the cell line used has not been specified in the legend text. This has now been corrected to state WM983B in the figure legend.

4. We agree with the reviewer that the effect of the combined treatment is weak in our in vivo experiments. As stated in our discussion: “...a weak increase in efficacy was observed in melanoma xenografts following combined treatment versus either mono-treatment alone.” The only significant finding was however the comparison of control and combination groups as indicated in the text (result part of manuscript). “Post-hoc comparisons using the Tukey HSD test indicated that the mean score of the control group (M=14.47, SD=2.87) was significantly different (p < 0.024) from the combination group (M=6.47, SD=3.88).” A sentence has been added to address toxicity in the xenograft experiments in the result section. “No direct immediate toxic effects were observed during the experiment with the given dosages.”

5. We agree with the reviewer that the routes of administrations of inhibitors in the in vivo experiments are not well described. We have therefore revised this paragraph in the Material and Method section and figure legend to include more detailed as suggested.
6. The reviewer pointed out poor quality of some western blot bands. We assume that the reviewer refer to Figure 4. A new blot has been enclosed showing a lower exposure to \(\gamma\)-H2A.X. The total level of Caspase 3 has been removed, and the figure now only shows the cleavage products that are indicative of apoptosis. We also agree with the reviewer that it is hard to see any additional effect of the combined treatment on the level of \(\gamma\)-H2A.X in the WM45.1 and WM983B cell lines. We have now emphasized this in the result section as well as made some changes in the discussion.

7. Several modifications have been included in the manuscript in order to improve the quality of the language as suggested by the reviewer.

**Reviewer Manmeet Raval**

1. The reviewer asked us to provide p-values for the differences among all combined and mono-targeting experiments. Statistical analysis of in vivo experiments has been included and discussed in the manuscript. Furthermore, we have conferred with several statisticians about inclusion of statistic data for all figures in the manuscript, who strongly discourage us from doing so. One concern is validity of such calculations due to few observations (n=3) and variability, suggesting that such p-values even though reaching 0.05 cutoff are highly unreliable and can be over interpreted. Since overuse of the statistic is discouraged, and including all the values does not influence validity or change the main conclusions drawn in the manuscript, we choose not to include these.

2. The reviewer comments that it would be of interest to know if lower doses of AZD7762 would give the same results as that provided in Figure 1C for WM983B cells. As a comment to this we refer to supplementary figure 2 where lower concentrations of AZD7762 were used, and a synergistic combined treatment effect was observed also in this cell line.

3. A relevant reference to a meta-analysis of median survival rate for patients diagnosed with distant metastasis of melanoma have been included.

4. The phrase has been corrected

5. The phrase has been corrected

All authors have read and approved the final manuscript.

Hoping that the manuscript will be accepted for publication in *BMC Cancer*, we look forward to your decision.

Vivi Ann Flørenes, Professor, PhD
Dept. of Pathology,
The Norwegian Radium Hospital, HF
Montebello,
0310 Oslo,
Norway

Tel +47-22 93 45 29
Email vivi.ann.florenes@radiumhospitalet.no