Author’s response to reviews

Title: PARP inhibition and the radiosensitizing effects of the PARP inhibitor ABT-888 in in vitro hepatocellular carcinoma models

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Author’s response to reviews:

Dear Madam, Sir

Please find the response point by point to the reviewers’ comments for our paper entitled “PARP inhibition and the radiosensitizing effects of the PARP inhibitor ABT-888 in in vitro hepatocellular carcinoma models” by Guillot et al.

Response to Reviewer no. 1

1. The authors show that the response of PLC-PRF-5 (with presumed lower PARP activity and lower capacity to repair defects) to combination of PARPi and IR followed a linear-quadratic model with radiosensitization starting at a higher dose of 3Gy than that of HepG2 cells, it would be better to have a direct comparison using the same model with the cells side by side

Radiosensitization, if any, will not begin abruptly at a certain dose of radiation but is a continuum depending on both alpha and beta parameters, and should be appreciated from the ratio of the D37 values (large difference for HepG2 cells, minor effect for PLC-PRF-5 only). To facilitate immediate, visual comparison of the two cell lines examined we have summarized the survival assay for both cell lines in a single figure in the revised manuscript.

2. No rationale is provided for the use of doxorubicin as a chemo sensitizer, is it possible that the differential response of the cells to PARP inhibition is reflective of differential response to anthracycline rather than PARP inhibition? As PARP trapping is likely the mechanism of action, would the results be different if the authors used a topoisomerase inhibitor?

Doxorubicin, which is topoisomerase II inhibitor, was not used as a chemosensitizer but as an agent to initiate pADPr synthesis in order to assess
the levels of PARP activity in the different cell lines (Figure 2D) and the effectiveness of PARP inhibition by ABT-888 (Figure 3A). The effects of PARP inhibition on cell survival were only assessed with ABT-888 alone or in combination with ionizing radiation.

3. We would advocate evaluation of caspase or other markers of apoptosis as a direct evidence of enhanced cytotoxicity of PARPi + IR, rather than indirect evidence using clonogenic assays as the authors have done.

We agree that assessing markers of apoptosis is important for a direct evidence of enhanced cytotoxicity of an anti-cancerous agent. However, it is recognized that apoptosis often plays a modest role in response to radiation exposure. Indeed, irradiation induces different types of cell death (mitotic catastrophe, necrosis, senescence, autophagy) with mitotic catastrophe being considered as the main type of cell death following radiation exposure especially in solid tumors that have often lost their pro-apoptosis mechanisms during tumour progression (Brown and Wouters (1999) Cancer Res 99: 1391-9). In addition cells die at various times after irradiation, often after one or two cell cycles, which is considered as a reproductive cell death (Endlich et al. (2000) Radiat Res 153:36-48). We thus choose to use the clonogenic survival assay to assess the cytotoxicity of our treatment strategy as this is a robust and relevant parameter to assess radiation effects, and considered as the “gold-standard” in radiation biology.

Response to Reviewer no. 2

1. The authors showed a significant correlation between PARP-1 and PARP-2 mRNA expression, or that of PARP-1 protein and mRNA expression. They should describe the information of correlation coefficient.

The Pearson correlation coefficients and p values were given in legends of figure 1 and 2 and this information has been added to the text now too. During the revision of the manuscript we noted a mistake in the r and p value of our tests and in the figure 2C; we have therefore corrected these values and the figure in the revised manuscript. The observed correlations are still statistically significant.

We thank the reviewers for interesting and constructive comments and do hope that the paper will be suitable for publication.

Best regards
Lyon 07.10.14

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