Author's response to reviews

Title: Pharmacological inhibition of poly(ADP-ribose) polymerase-1 modulates resistance of human glioblastoma stem cells to temozolomide

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Author's response to reviews:

Dr Francois Ducray
Editor of BioMed Central
Re: MS: 9456903631107536

Dear Editor:

Thank you for the interest in our work. All referee’s comments have been carefully evaluated and the manuscript has been modified accordingly.

Referee 1

(i) The PARPi used in our study, GPI 15427, and its analogue of E7016 have shown ability to cross the blood-brain barrier and chemo- radiosensitizing activity in preclinical models of glioblastoma (see last paragraph of page 18 of the revised manuscript and references 10 and 37). E7016 is currently under phase I clinical investigation in association with TMZ for solid tumors, including glioma (phase I, NCT01127178), and for metastatic melanoma, including patients with brain metastases (phase II, NCT01605162) (see inclusion criteria of both clinical trials in www.clinicaltrial.gov) (last line of page 18 and first line of page 19 of the revised manuscript).

(ii) PARP inhibitors have demonstrated synergy when combined with methylating agents against melanoma (temozolomide and dacarbazine) and glioblastoma (temozolomide). The mechanisms underlying the synergy between PARPi and temozolomide have been described by ourselves and several other authors (reviewed in ref. 5 and 12). Briefly, PARP-1 plays a key role in the repair by the
base excision repair (BER) of N-methylpurines (N7-methylguanine and N3-methyladenine) that are generated by temozolomide. In the presence of a functional BER system these damaged bases are promptly repaired and do not contribute to temozolomide cytotoxicity. Therefore, the enhancing effect exerted by PARP inhibition on temozolomide antitumor activity is the consequence of increased DNA damage that eventually results in apoptosis and/or growth arrest (see page 5, lines 10-16 of the revised manuscript).

(iii) As requested, we provided details on the GSC markers, the methodology and antibodies used for their detection (page 7, lines 11-24). Moreover, we removed the reference N. 20 of the original manuscript that referred to the in vivo ability of GSC to form glial tumor in immunodeficient mice, since the referee asked to remove the unnecessary sentence about the in vivo tumorigenicity, and we replaced it with a more appropriate reference (reference N. 20 of the revised manuscript Ciceroni C et al. Cell Death Differ 2013, 20:396-407, that includes the methods for detection of SOX2 and Musashi-1. Nevertheless, the gold standard to classify a cell as a glioma stem cell is that it can form a xenograft tumor that is capable of serial transplantations in immunodeficient mice and all GSC lines used in this study are tumorigenic in vivo. In regards to stem cell markers, there is currently no universally accepted collection of markers for isolation of a pure population of glioma stem cells (J Cell Biochem. 2009;108:1031-8. doi: 10.1002/jcb.22350.). The heterogeneity of malignant gliomas renders difficult the use of a single set of markers to identify glioma stem cells. In particular, CD133 is not considered an universal stem cell marker for GBM. Moreover, all of them were positive for glioma the stem cell genes, Sox2, Nestin and Musashi-1, whereas variable percentages of CD133 were observed (page 7, last three lines). In the revised manuscript, when not clear, we specified whether GBM cell lines were included in the statistical analysis (see page 13, line 22-23; page 14, line 15).

(iv) We agree with the referee that it cannot be excluded that the mutation status and copy number changes of PTEN might have different roles in the sensitivity to PARPi. Thus, in the revised manuscript a sentence was added on this issue (see page 18, lines 12-15).

Minor points:

- In the statistical analysis paragraph we specified that, for multiple comparisons, the Anova was followed by Bonferroni’s post-test and that the non-parametric Kruskal-Wallis analysis was followed by Dunn’s post-test (see materials and methods, page 10, last 3 lines, and Results page 14, lines 20-22). Thus, for multiple comparisons, the presented P values were always adjusted.

Thanking you and the referee for giving us the opportunity to improve our manuscript, we hope that the revised manuscript will be now suitable for publication in BMC Cancer.
Looking forward to hearing from you soon,
sincerely,
Lucio Tentori