Reviewer's report

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

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Reviewer: Agustí Alentorn

Reviewer's report:

This is an interesting article showing that the combination of a PARPi (analogue of E7016) with Temozolomide may have a synergic effect in vitro (in GSC). This article is well-written and data is sound. However, the interpretation and clinical relevance of these findings are not properly discussed in this paper.

There are other major compulsory revisions that should be clarified throughout this paper:

(i) More insight about the in vivo effect of this inhibitor is needed to demonstrate that a correlation between in vitro and in vivo exists. For example, it is unknown whether the PARPi used may cross the blood-brain barrier and whether this pharmacological synergism also exist in vivo. In this line, in the article authors say that there is one Phase I trial ongoing to test this association of PARPi with TMZ for solids and gliomas. According to clinicaltrials.org, this reviewer has only been able to find one Phase I study (NCT01127178) and one Phase II study (NCT01605162) using E0716, but none of them include primary or secondary brain tumors. Could authors provide the reference of such trial?

(ii) Authors should provide more insight about the mechanisms that may facilitate or promote this synergism between TMZ and PARPi, maybe by using high-throughput approaches like transcriptomic analysis of cell lines in order to identify pathways that may be involved in such effect.

(iii) Authors should provide more details on how they have defined and used stem cells markers. In cell cultures paragraph, it is stated that "cell markers such as CD133, SOX2, Musashi and nestin, capacity of self-renew, ability to co-express astrocytic as well as neuronal phenotypic markers after serum-induced differentiation in vitro, and generation of glial tumors in immunodeficient mice [20,21]". As this is an in vitro study, this paragraph is of paramount importance in order to reproduce/validate these results. Firstly, as there is no animal model or in vivo test in the paper, last sentence should be deleted. Secondly, it is not clear if all markers should be present or only the combination of some of them is needed to classify neurosphere as a GSC. In addition, in the references provided do not use all these stem cell markers. Moreover, authors use two cell line (not GSC) in most of the tests, however it is not clearly stated when these cells lines have been included in the statistical analysis and when not (for example n values of Spearman’s correlation test
should be rechecked). Finally, all the antibodies used for the detection of stem cell markers are not provided.

(iv) Authors state that a possible mechanism to explain the low number of GSC that express PTEN could be related to the high frequency of PTEN mutations / losses at 10q23 or by other mechanisms of PTEN inactivation in GBM. However, these analyses are not performed in these GSC and the mutation status and copy number changes in PTEN may have a different role when considering PARPi activity.

There are minor essential revisions that need to be clarified:

In statistical analysis paragraph it is stated that Bonferroni’s correction and Dunn’s test are used for multiple comparisons adjustments. However, throughout the text should be clearly stated whether the presented p-values are the adjusted ones or not. In correlation analysis the sentence is confusing, “correlation analyses were performed using the Spearman’s rank test and significance was determined according to” p-values (not to the correlation coefficient).

**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