Reviewer's report

Title: BRCA1 promoter hypermethylation, 53BP1 protein expression and PARP-1 activity as biomarkers of DNA repair deficit in breast cancer

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Reviewer: Christos Kroupis

Reviewer's report:

General remarks for the paper:

Authors have tried to investigate the use of the following three biomarkers: cPARP1 enzyme activity, BRCA1 hypermethylation and 53BP1 protein levels in the very interesting question of finding better selection criteria for PARP1 inhibitor therapy than the criteria of triple-negative (TN) status. The PARP1 enzyme activity did not show any promise towards that goal. For the remaining two, the theoretical concept is that besides the BRCA1 mutant patients (that are not addressed in this study), only sporadic breast cancer patients showing homologous recombination deficit (as indicated by BRCA1 hypermethylation) and NHEJ activation (as indicated by high levels of 53BP1 protein levels) could benefit by PARPi therapy. In their group of 155 sporadic patients only few fit this dual demand (the above principle remains to be tested in a future follow-up study).

Major Compulsory Revisions

1) In order to be more accurate, authors have to acknowledge the limitations of their interesting approach: a) BRCA1 hypermethylation tested by methylation-specific PCR (MSP) in the area under the two primers is only an indication of BRCA1 silencing especially in the cases of weak signals: pyrosequencing would be more thorough and quantitative, also in the absence of good mAbs to assess quantitatively BRCA1 protein levels, BRCA1 mRNA levels would most probably present a more accurate biomarker b) their sporadic patient group -although without evident family history- could still hide few BRCA1 mutants (we do not know how thoroughly they were tested for BRCA1 mutations) especially in young patients or even mutants in other less common homologous recombination genes with moderate penetrance (e.g. PALB2, BRIP1 etc). PALB2 mutations can also be PARPi targets as shown in Buisson R et al. 2010, Hellebrand H et al, 2011, Poupouridou et al., 2012. Hypermethylation in these genes could also be indicative of homologous recombination deficit in “true” sporadic cases.

2) In order to have equal numbers in the three molecular profiling groups, authors have selected practically only advanced grade (II and III) patients (only one grade I patient exists in their group). This has to be clearly mentioned in the abstract text and in the beginning of the discussion (is it also really necessary to mention non-consistently the term EE-SBR instead of grade -except for the first
time that is mentioned-?)

3) An extra table should be provided for the 53BP1 protein results since these are the main findings of this study (a suggestion in order to keep the number of tables the same, table 1 could probably be omitted if the Esteller MSP primers are used in this study, if not, please provide also the location of the primers in reference to GenBank).

4) Regarding statistics, the correlation of the number of mitoses with cPARP-1 activity is weak (not strong as indicated, 0.234) but significant (p=0.003). From suppl Figure 1, from the data r²=0.0065 we can conclude that there is no correlation between 53BP1 levels and PARP1 activity. In page 13, what do you mean exactly with the 1% threshold?

5) In page 12, it is mentioned that both high 53BP1 levels (above 9.6 pg/mgP) and BRCA1 hypermethylation occur in four TN tumors (however in Suppl Table1: three) and one non-TN tumor (in Suppl Table1: two in the two other MPGs).

6) Since the uPA/PAI-1 and LVI results are not particularly discussed or in other words, they are not the focus of this manuscript, it is highly suggested that they are omitted. When the processing of the tissues is first mentioned in Methods, please cite your corresponding reference [ref. 39].

7) Suggest avoiding the terms pathway and networking for the interplay of the 3 tested biomarkers because these markers do not belong in the same pathway.

Minor Essential Revisions

8) Have authors used any negative and positive controls in the BRCA1 MSP reactions?

9) Please mention the amount of protein used in each sample for 53BP1 testing, the mAbs used for ER and PR IHC and the composition of the Triton buffer in the tissue homogenization.

10) According to table 2, correlations with PARP1 activity were performed with the use of the median. Were they also performed with the mean or the upper quartile limit as cut-off values as it is mentioned in the PARP1 results section?

11) Are BRCA1 hypermethylation and BRCA1 mutations mutually exclusive as mentioned in page 5?

12) Please fill info for references 43 and 44 and for Qiagen and Eppendorf companies in the Methods section please mention their true country of origin.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.