Reviewer's report

**Title:** Oxidative stress and counteracting mechanisms in triple-negative and basal-like breast carcinomas

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**Reviewer:** Felipe Geyer

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In the present manuscript, Karihtala et al. aimed to investigate whether or not oxidative stress and/or cell redox state-regulating enzymes have special roles in triple negative and basal-like (BLBCs) breast cancers. To address this question the authors assessed the immunohistochemical expression of 8-OHdG, Nrf2, Keap1, PRDX III and PRDX IV in a small series of 79 invasive ductal carcinomas, including 37 triple negative tumours, of which 12 were classified as BLBCs. This is a simple study with few significant findings, mostly related to non-triple negative tumours despite the title of the paper. Association with survival independent of other prognostic factors has not been demonstrated, limiting the significance of the results. Below are specific comments:

**Major Compulsory Revisions**

1) It seems that the authors misunderstood the Nielsen immunohistochemical surrogate panel for BLBCs. According to Nielsen et al. (Clin Cancer Res 2004) and Cheang et al. (Clin Cancer Res 2008), BLBCs are defined as triple negative tumours expressing EGFR and/OR CK5/6. Therefore, to classify BLBCs as “the triple negative tumours that also expressed both EGFR and CK5/6” may be incorrect and is certainly not very sensitive, considering that the panel as described by Nielsen et al. has a sensitivity of 76%. Moreover, requiring expression of both EGFR and CK5/6 would not increase the specificity, which is 100% when considering EGFR and/or CK5/6. This should be amended in the study and all analysis comparing the BLBCs re-done.

2) There is large amount of evidence to suggest that at least the great majority of BLBCs do not originate “from stem cell precursors of basal myoepithelial cells”, but actually from ER-negative luminal progenitor cells. Please see Molineux et al. Cell Stem Cell 2010 and Lim et al. Nat Med 2009 and amend the introduction.

3) In 2010, the ASCO/ CAP guidelines for evaluation of ER and PR were published (Hammond et al J Clin Oncol 2010). According to those guidelines, a cut-off of more than 1% (and not 10% as used in the present study) of positive cells should be used for considering a tumour ER/PR positive. Therefore, the study has not been “updated to match the latest criteria concerning ER- and PR-negative tumors”. This should be updated in the manuscript.

4) How specific and sensitive are the antibodies used in the study? The authors failed to demonstrate how were the not commonly used antibodies PRDXIII, PRDXIV, 8-OHdG, Nrf2 and Keap1 optimised. What has been used as positive
controls? Adequate optimisation and positive controls ought to be demonstrated as this is of utmost importance for an immunohistochemistry-based study.

5) Expression of both 8-OHdG and Keap-1 were significantly associated with prognosis. Given that both markers were significantly associated with other prognostic factors, such as histological grade and triple negative phenotype, the authors ought to perform a multivariate analysis and describe the results in the manuscript.

6) The authors mention in the discussion that “8-OHdG was a marker of good BCSS also in the current population and this difference was similar in all studied subgroups”. Data in relation to the distinct studied subgroups has not been provided in the manuscript. Considering the small number of cases in the subgroups, it is unlikely that the differences are significant.

7) The title and the conclusion of the abstract are misleading. The title should mention breast cancers in general as the positive findings of the study and most of the discussion are in relation to ER+/PR+/HER2- tumours. Moreover, it does not seem to this reviewer that the results of this study have helped “elucidating the pathogenesis of TNBC”. Although the abstract conclusion may indeed hold true, this has not been demonstrated in this study. Likewise, the conclusion of the manuscript does not match with its title.

Discretionary Revisions:

1) If the rationale that oestrogen induces oxidative stress and anti-oxidant defense via Nrf2/Keap1 pathway is correct, one should expect higher nuclear expression of Nrf2 in ER+/PR+/HER2- tumours. However, no difference was detected in the levels of expression of Nrf2 between ER+/PR+/HER2- tumours and triple negative tumours. It would be interesting to discuss those contradictory findings.

Level of interest: An article of limited interest

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.