Reviewer's report

Title: Nucleostemin expression in invasive breast cancer

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Reviewer: Caterina Marchiò

Reviewer's report:

The paper by Kobayashi and colleagues investigates Nucleostemin as a potential prognostic factors in breast cancer. The results are potentially interesting, since the prognostic relevance is maintained also in the multivariate analysis, however some points need clarification.

Major compulsory revisions:

- about the cohort: the analysis was conducted in a cohort of 220 carcinomas, but it is not clear whether this was a consecutive series or there had been a selection of cases. In addition, the patients have not been treated homogeneously, so this is a bias and should be mentioned in the discussion as a word of caution. Finally the ER, PR, HER2 were taken from records of a previous study, but it is important to clarify whether these markers were re-assessed or not in order to have a more homogeneous evaluation: what were the cut-offs for positivity for ER ad PR? The cases belong to the '90, when HER2 was not an established routine as yet, therefore was HER2 performed on new sections? Lastly, why ki-67 has not been performed?

- about the IHC of the marker: it is not 100% clear whether Nucleostemin is positive also in the normal tissue, the authors state in the introduction that nucleostemin not in the differentiated somatic cells of most adult tissues, however at some point in the results they state "Unremarkable mammary glands showed nuclear NS immunoreactivity in almost all luminal epithelial cells": are they referring to unremarkable CANCER mammary glands or to normal mammary gland? Figure 1D is the reference for this statement, however from the area they took it is not trivial to understand whether it is normal breast or cancer, as the illustrated structures are glandular but a bit distorted and the field is not very representative, it is difficult to figure out. Please clarify this point.

Was nucleostemin correlated in particular to any histological type?

- about the prognostic relevance of nucleostemin: curiously, two strong prognostic factors have not been taken into account in this analysis, however I believe they shouldl, namely the proliferation index (as also mentioned above) and the histological grade. Correlations should be performed with these two parameters and the multivariate analysis run again.

Minor essential revisions:

- the authors inappropriately use some adjectives: for example "breast cancer is
one of the most serious and prevalent diseases worldwide": serious disease is not very scientifically sound.

- is the formatting of the manuscript in accordance with the Journal's guidelines? Please double check.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests