Reviewer's report

Title: Relationship between circulating tumor cells and epithelial to mesenchymal transition in early breast cancer.

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Reviewer: Vera Cappelletti

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REVIEWER’s REPORT
The manuscript by Mego et al presents a well-written study, which builds on robust data. However, the authors did not finalize their study, which makes it weak from a translational perspective. Nonetheless, in the manuscript the authors define their study as ‘translational’ adding confusion.

The role of CTC in early breast cancer definitely merits to be further explored, but the simple observation of patients with pure epithelial, mesenchymal or mixed CTC does not represent a novelty: again, the translational relevance is not clear. Indeed, the abstract itself lacks an informative conclusion and simply underlines the need of future studies.

Major compulsory revisions:

Results
This referee is a bit confused with the reported numbers: CTC are claimed to be present in 24.5% of patients, but when detailed, the authors report 11.8% of CTC with epithelial only features, 14.7% of patients with mesenchymal CTC and 2% of patients with CTCs co-expressing both markers. The sum comes to 28.5 and not 24.5.

Line 144: probably the Authors did mean ‘identification of gene transcripts in CD45-depleted subsets’

Material and methods
No details on the stroma sampling are reported. How far was the stroma used for IHC from the tumor lesion?

It is very important to define the timing of blood withdrawal for CTC. Was it pre- or after surgery?

The positivity criteria for defining the CTC status need to be better described. Is expression of just one of the markers (either epithelial or mesenchymal) at levels above the defined cutoff enough to define a sample as CTC positive?

No data are reported on the retro-transcription preventing from understanding the amount of RNA from which the assay started

Discussion, line 299: the explanations reported for the lack of correlations between IHC data and CTC should be considered as study limitations rather than
true explanations.

Conclusion: This referee feels that the conclusion reported at the end of Discussion: ‘these results suggest that expression of EMT proteins in unselected tumor tissue is not a surrogate marker of tumor invasivity and its metastatic potential’ is not supported by the data. In fact evaluated biomarkers (CTCs and tissue EMT proteins) were neither correlated with any clinical outcome (as it is probably not available for this patient series), nor CTC status (as evaluated in this study) can be considered a surrogate marker of outcome.

The authors claim the need of future studies to identify ‘expression of proteins in tumor tissue associated with presence of CTC in peripheral blood’. Since studies reporting CTC status/enumeration and gene expression profiles are available in the literature (e.g. Molloy et al PlosOne 2012 7, e32426), this referee would suggest to use them for a preliminary exploration of tissue genes associated with presence of CTCs, possibly separately evaluating early and metastatic tumors.

Minor revisions

Line 125: the sense of the sentence ‘no children, a parent or guardian were involved into the study’ is not clear.

How many pathologists were reviewing samples? Contrasting data are reported, see line 175 vs line 210line

Lines 240-242, please rephrase to make the sense clearer

Line 308, please correct: ‘didn’t observed’

In the tables report the statistical test used for calculating p-values.

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