Reviewer’s report

Title: Co-expression of putative stemness and epithelial-to-mesenchymal transition markers on single circulating tumour cells from patients with early and metastatic breast cancer

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Reviewer: Giorgio Stassi

Reviewer's report:

Although the subject of this proposed manuscript is potentially interesting, the data presented are not sufficient to speculate general conclusions. Moreover, an EPCAM+CD44+CD47+MET+ subpopulation has been already identified in breast cancer patients. In particular, these metastasis-initiating cells was able to give rise to a fenocopy of the parental tumors and were correlated with a worst prognosis in a cohort of metastatic breast cancer patients. The manuscript data are incomplete and based only on a simple identification and characterization of ALDH1 and TWIST expression in CTC from breast cancer patients.

Major concerns:

1) According to the Epithelial-to-Mesenchymal transition (EMT), a cancer cell acquires the mesenchymal trait in order to give rise to a metastasis. For this reason authors identified a TWIST nuclear localization principally in CTC of metastatic patients. This is in contrast with the staining of the same cells with anti-pancytocheratin. The analysis of TWIST localization is not sufficient to support the authors conclusions. Other mesenchymal markers should be investigated.

2) In order to identify a subpopulation with a metastatic potential authors should perform an in vivo model in which CTC co-expressing ALDH and TWIST are able to give rise to a tumor that resemble the parental.

3) Authors reported that all cell lines showed an heterogeneous population for both ALDH1 and TWIST stainings. Considering that the cell lines analyzed are representative of the three different breast cancer subtypes: luminal, basal and HER2 pos; data obtained do not provide any novelty. Moreover, all the images should be showed with a lower magnification.

4) Results reported in the text do not always correlate with the images. For example, authors report that HepG2 cells have a TWIST nuclear localization while in the Figure 1 it seem cytoplasmatic. Authors should better check the correlation between Figures and text.

Minor revisions

1) Several grammar errors
Level of interest: An article of limited interest

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I have not any competing interests in relation to the paper that I am reviewing.