Author's response to reviews

Title: Mesenchymal and stemness circulating tumor cells in early breast cancer diagnosis

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Author's response to reviews:

Reviewer1
As asked our manuscript has been corrected by a English native scientist.

Reviewer 2
1. In the new manuscript we described more accutely the method. It is still summarized as we refered to the publication of Aktas et al and Banys et al, where it is totally described. We cited Aktas et al who published evidently one of the first paper dealing with this subject.

2. For detection of CD44 and Bmi we detail informations about single-plex RTPCR particularly we inform about primers which have been used. We supply the number of normal donors tested to establish a cut-off of these markers. This was indicated in the first manuscript and perhaps the rewiever did not noticed the sentence about this information. It was suggested by the reviewer, that the positive rate of 67% for Bmi1 is too high and implicated high number of false positive samples. If we considered that 39% of our patients are positive for ddCTC, 67% indeed means that upon 24 positive patients 16 expressed the BMI stemness marker. It is really normal that cells being characterized by EMT and stemness markers expressed the Bmi1 factor which is known as the one involved in the maintenance of stemness status. Moreover enrichment was made by the EMT select kit of Adnagen (and not with the breast cancer select kit) leading to a decrease in the number of contaminating leucocytes.

3. We do not understand why statistical analysis is not feasible with 61 patients, the three test used were realized by an expert statistician and moreover the results agreed for the three tests. To confirm this statistical analysis the study is pursued awith more results in sight.

4. The discussion has been reevaluated taking into account most of published results. We do not think that figure 2 should be deleted as it explains EMT and the choice of markers.
5. In their publication Braun S et al. (Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med 2000, 342: 525-533.) studied disseminated tumor cells (micrometastases in the bone marrow) and established that absence of lymph node invasion is not a criterion of non-dissemination. We agree with this assertion. Our results suggested a similar approach: presence of ddCTC is correlated to absence of lymph node invasion.

6. To make this paper suitable for publication it was corrected by an English native scientist.