Reviewer’s report

Title: Isolation and Genomic Analysis of Circulating Tumor Cells from Castration Resistant Metastatic Prostate Cancer

Version: 1 Date: 1 December 2011

Reviewer: Thomas Höfner

Reviewer's report:

Major Compulsory Revisions:

The authors did not reply sufficiently to the aspects the revision stated before, in particular the following has to be changed in order to accept/publish the manuscript:

1. The introduction/discussion does not include enough critical remarks about the ongoing controversy about the relevance of CTC due to missing xenograft/functional studies. It is neither a functional proof to provide CGH analyses, nor does it answer relevant questions regarding this controversy. It is therefore not important to further characterize these cells before it is clear and biologically proven that they are functional and relevant for the metastatic cascade. That’s the point many authors did before and this relevant discussion, specifically the non-existing multivariate analyses of prostate cancer CTCs together with currently used clinical nomograms (like the Stephenson nomogram) has to be pointed out more clearly. It is not sufficient to add only one sentence. This should also be done to push the field more towards clinical relevance. We do not need more molecular profiling before basic biological questions were answered sufficiently.

2. The separation and detection method of CTCs by magnetic enrichment/FACS is not new, this assumption of the authors remains wrong (e.g. Racila et al, PNAS 1998: Detection and characterization of carcinoma cells in the blood), maybe the authors use a different antibody for EpCAM or include for the first time a different CD45 antibody compared to studies before (like PCR analyses after FACS sorting of CTCs, there are numerous published), but this specific minor novelty has to be pointed out compared to studies using FACS in CTC analyses before. The whole FACS sorting approach and subsequent CGH cannot be regarded as novel method. It combines two separate known techniques.

Are the methods appropriate and well described?
3. The methods/results are still not described appropriately. The provided FACS plot by the authors is by far not enough to provide reliable data.
- as argued before it is important to demonstrate FACS plots before and after sorting the CTCs, at least for 2 of the included patients to ensure subsequent CTC analyses.
- it is essential to include a dead cell exclusion dye before CTC sorting, this has to be demonstrated (7-AAD or propidium iodide) otherwise we do not know if the cells were dead before they were sorted.
- All FACS analyses/plots have to be correctly compensated. If the pictures in supp. fig 1 show semi-logarithmic curves, this has to be mentioned and at least examples have to be provided how the signal really behaves. The reader has to understand the analyses. If only a diagonal can be seen between PerCP-Cy5.5 and PE I would argue against correct compensation of fluorescent spectra. In this case gate P3 would either have missed allmost all positive EpCAM –cells or all the cells were CD45 positive.
4. Please explain, how exactly do you correctly microdissect the cancer area with a surgical blade after you analyzed H&E slides? Prostate cancer is known to be microscopically multifocal... this is not manageable and wrong. The authors should include a sentence in the discussion, that there are limitations for genetic comparison without e.g. the use of laser microdissection.

**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