Author's response to reviews

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

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Author's response to reviews: see over
Dear Members of the Editorial Board:

We are submitting our revised manuscript by M.J.M. Magbanua, E.V.Sosa, J.H. Scott, J. Simko, C. Collins, D. Pinkel, C. Ryan and J.W. Park entitled:

**Isolation and Copy Number Analysis of Circulating Tumor Cells from Castration Resistant Metastatic Prostate Cancer**

that we wish to have considered for publication as a Technical Advance in *BMC Cancer*. We include in this submission our point-by-point response to the reviewer’s concerns.

If you have any questions, please do not hesitate to contact me at any of the following: Tel.: (415) 502-3844; Fax: (415) 353-9592; email: jpark@cc.ucsf.edu

Thank you very much.

John W. Park, M.D.
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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.

- We have added the following to the Introduction section:

The biological significance of CTCs remains unknown, underscoring the need for functional analysis and molecular characterization of these cells. Furthermore, the role of CTC detection in the clinical management of prostate cancer remains debated. For example, the relation of CTCs to currently used diagnostic tools, such as clinical nomograms, is unclear.

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.
- We have added the following to the Discussion section:

*Our approach involving immunomagnetic enrichment, FACS sorting and aCGH analysis is novel, and this report confirms the feasibility of this approach for genomic analysis of isolated CTCs. Immunomagnetic enrichment alone provides a cell mixture which is predominantly hematopoietic, although further analysis may be performed on the admixture of cells [1-3]. In a notable prior report, Racila et al. described immunomagnetic enrichment followed by flow cytometry for enumeration of CTCs, as well as enrichment followed by cytospin for immunostaining [4]. Our approach enables full FACS-based isolation of CTCs for molecular profiling.*

3. Are the methods appropriate and well described? 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.

- We have added the following to the Methods section:

*Due to the small numbers of CTCs isolated from patients' blood, it was not feasible to perform FACS analysis after sorting.*

- In addition, we have revised Supplementary Figure 1 to include two representative patients, as suggested by the reviewer.

- 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.

- We have added the following to the Methods section:

*Blood samples were collected in CellSave™ (Veridex) tubes [5-6]. CTCs were thereby rendered nonviable and fixed to maintain antigenicity prior to immunomagnetic enrichment and FACS sorting.*

- 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 2 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 almost all positive EpCAM –cells or all the cells were CD45 positive.
- We have revised Supplementary Figure 1 to include two representative patients, as suggested by the Reviewer. We have also added the following text to the Supplementary Figure 1 legend:

*Forward scatter (FSC) vs side scatter (SSC) plots are on a linear scale while SSC vs nucleic acid dye plots are on a semi-logarithmic scale. EpCAM-PE vs CD45-PerCP-Cy5.5 and EpCAM-PE vs nucleic acid dye are log-log plots.*

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.

- We have added the following to the Methods section:

*In the case of a small tumor (Patient #20), manual microdissection of the selected area was performed by scraping successive tissue sections under a stereomicroscope.*

- We have also added the following to the Discussion section:

*Since prostate cancer is multifocal [7], the use of laser microdissection to isolate different clones present in the primary tumor will further refine our understanding of the clonal relationships of CTCs and their primary tumor focus of origin.*

**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
References:


