Reviewer’s report

Title: CD133 expression is not an independent prognostic factor in stage II and III colorectal cancer but may predict the better outcome in patients with adjuvant therapy

Version: 6 Date: 14 January 2013

Reviewer: CLAUDIU MARGARITESCU

Reviewer’s report:

Discretionary Revisions

Overall, the role of a reviewer is to bring constructive ideas and perspectives to strengthen a study, and in this line I consider that the present study can be published, leaving as an option for the authors to include and discuss my present comments.

2A. Regarding our initial comment, the puzzling fact is that although the literature discusses different epitope availabilities (thus maybe different tertiary and quaternary conformations after some hypotheses rather than glycosilation) during tumor cells differentiation, the existence of positive non-stem tumor cells, as well as the different pathological types and subtypes of colon tumors; your results still show a correlation with the mRNA levels without classifying the data in these categories (Kemper K at al., 2010). So it is pertinent to ask if classifying the data (with double IHC mainly) would show in fact different correlation degrees for different cell compartments or tumor types.

2B. At this point, as a pathologist, I still believe that for poor differentiated tumors it is a difficult task to compare IHC slides versus HE even on consecutive slides (and basically being asked to identify the same cell in both sections), to establish the epithelial/non-epithelial origin of the CD133 signal.

2C. Here I cannot agree to the authors in what it regards the use of image analysis software. In almost any good image analysis package, the investigator can decide what areas of the image to be analyzed. That is to define the region of interest (ROI) that would be further analyzed. So after the trained pathologist define the ROIs on the image a macro function can be implemented to measure the signal in that area (integrated optical density for example). Together with a more precise area estimation (by aligning the software with the microscope’s objectives), this type of analysis would be much more recommended for this study, especially considering the discussion of the epitope aviability variation. A two-investigators blind study is effective here too in defining the ROIs and creating the RGB profile of the signal to be analyzed.

3. Here I indeed meant the dissection of single cells, which from experience, is possible and feasible especially for this type of analysis.
References:

Level of interest: An article of importance in its field

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.