Reviewer’s report

Title: Immunohistochemical analysis of cancer stem cell markers in locally advanced pancreatic adenocarcinoma patients after neoadjuvant chemoradiotherapy

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Reviewer: Wenqing (Wendy) Cao

Reviewer’s report:

The manuscript by Mizukami et al. tried to evaluate the effect of neoadjuvant chemoradiation therapy (NACRT) on expressions of pancreatic stem cell markers using the immunohistochemistry method. In the study, pancreatic CSC markers including EpCAM, CD44, CD24, CD133, CXCR4 and ALDH1 were studied in a total of 28 pancreatic adenocarcinoma cases (17 cases with NACRT and 11 cases without NACRT). The authors demonstrate that the frequencies of expression of CD44 and ALDH1 are higher, whereas CD133 expression is lower in NACRT group compared to non NACRT group. They also show that CD133+/ALDH- expression in NACRT group is associated with unfavorable patient outcome. These results seem to support the theory that radiation and/or chemotherapy may alter the expression of cancer stem cell markers. One recent report has shown that neoadjuvant chemotherapy with gemcitabine increases CD44 expression in pancreatic adenocarcinoma (Tajima Oncology Letters 2012). Some data in the study are interesting and may have certain impacts on pancreatic adenocarcinoma patient treatment and prognosis. However, there are several major concerns regarding the experimental design and data interpretation:

Major Compulsory Revisions:

1) This is a small sample size retrospective study done only in 17 cases in NACRT group. There are only 1 or 2 cases in many groups listed in Table 4 and 5. It is highly recommended the authors to expand their sample size.

Minor Essential Revisions:

2) When evaluating IHC expression, the authors used two to six visual fields to evaluate staining intensity and estimate the fraction of positive stained tumor cells. How did the authors define the visual fields and how did they apply the visual fields to different markers? Please explain.

3) Most pancreatic cancer patients are resistant to chemoradiation therapy. It would be interesting to see some kind of association between stem cell marker expression and treatment response. Although the authors showed in Table 5 that there are no significant differences between stem cell marker expression and treatment response, this might be biased by small sample size.

4) Many results in the current manuscript appear to be inconsistent with the
published data. Authors did not interpret and discuss them. These discussions may make the study more significance to the audience.

**Level of interest:** An article of importance in its field

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

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests