Author's response to reviews

Title: Platinum sensitivity and CD133 expression as risk and prognostic predictors of central nervous system metastases in patients with epithelial ovarian cancer

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Author's response to reviews: see over
Letter addressing reviewers' comments

Dear Editor,

On behalf of all authors of this manuscript, I would like to thank the reviewers for helpful and constructive comments. Below are our responses to each comment.

Editorial Request:

“Please include the name of ethics committee that approved your study.”

We have now included the name of ethics committee list it here that approved our study.

Reviewer #1:

1. “The question posed by the authors is relatively well designed but it remains unclear as to why CD133 was chosen as a risk factor for CNS metastases and relevance to such a presentation.”

Several studies have shown that CD133 expression is correlated with prognosis in primary brain tumor patients including astrocytoma, oligodendroglial tumors, neuroblastoma, ganglioneuroblastoma and gliomatosis cerebri but to our best knowledge, CD133 expression has not been evaluated in patients with CNS metastases. We have now modified the introduction paragraph (p. 6) to clarify this point.
2. “The methods are clear but the glaring question remains as to how the expression of CD133 could differentiate between patients with recurrent ovarian cancer with or without CNS metastases. To that end the analysis would have been stronger if each patient would have had a number of similar controls without CNS metastases to be able to compare the risk associated with CD133 expression”

We have now included a control group of EOC patients without CNS metastases and compared CD133 expression between the two groups.

The paper abstract, methods, results, discussion and conclusions were updated to include these additional data.

3. “The data is relatively sound but would have been more compelling had the design been a case control study since this is a small cohort of patients with variable CD133 expression when multivariable analysis is at best weak since most subgroups contain 10 or less patients. Table 2 is confusing with tiny numbers in each cluster which is weakens the strength of the associations, I would have preferred separated columns for the p value but also doubt the utility of such table in light of it's weak statistical importance. A table outlining the correlation between primary and metastatic sites with regards to CD133 expression would have been preferable.

Also I would have liked to see more details about the neurologic symptoms
the authors allude to.”

The points raised by the Reviewer are addressed in sequence below (A, B and C):

A. We have now modified the old Table 2, shown as Table 3 in the revised manuscript and used separated $P$ values to illustrate the difference between groups with CD133- expression vs. CD133+ expression. The statistical power is relatively sufficient (close to 0.80) even considering a relatively small sample size.

B. We have now added a table (new Table 4) to show the correlation between primary and metastatic sites with regards to CD133 expression as the Reviewer suggested. We have also included the results in the revised manuscript (p. 11).

C. We have now included the information on neurologic symptoms reported in patients with CNS metastases in the Results section (p. 9).

5. “The discussion and conclusion are clear but suffer from the lack of comparison to patients without CNS metastases which could have strengthened the conclusion and this should have been included among the limitations of the study which are otherwise clear.”

We agree and have now included a control group of recurrent EOC patients without CNS metastases. These data have now been addressed in the Discussion.
Reviewer #2:

1. “This article by Liu et al. demonstrates following findings through an analysis of 29 epithelial ovarian cancer (EOC) cases with CNS metastases-
   1. Extent of surgical excision and platinum sensitivity of EOC are associated with time to develop CNS metastases and
   2. CD133+ cluster formation in CNS metastases and application of multimodal treatment are respectively prognostic factors

They analyzed a pretty large cohort with more than 1300 EOC patients, which would provide a clinically significant evidence although it was a single-institute retrospective study. As for the 1st issue, these two are general prognosis predictive factors as authors described. Brain metastasis is itself very rare, but in this context, it appeared one of consequent recurrent places resulting in reasonable poor outcome in those with CNS-metastasis. And a systematic review for 591 patients bearing brain metastasis from EOC was already published by Pakneshan et al., and the efficacy of multimodal treatment has been reported in several reports. Thus, this reviewer should state that the findings authors confirmed are valuable but not novel in terms of the significance to provide a new implication in the management of EOC.”

We have now quoted the systematic review published by Pakneshan et al. and we agree that parts of our data are in keeping with the findings of
theirs and several previous studies. Moreover, our results point to CD133 as a potentially important player in tumor metastasis also there are, no doubt other players (as these data suggest – CD133- patients also got brain metastasis). Having an easily measurable biomarker associated with the metastatic disease may allow this population of patients to be optimally screened and improve disease outcomes.

2. “Authors mentioned the facts that cisplatin was not able to overcome the blood-brain-barrier, and that CNS-metastasized tumors mainly consist of lung or breast cancers. Readers including this reviewer would like to know the potential markers in primary EOC to indicate future cisplatin-refractory CNS-metastasis. The marker might be one of molecules or transcriptional factors (TF) associated with aggressive features of breast cancer or lung cancer. In this genome-wide analysis era, several datasets of breast cancers and lung cancers consisted of cases with or without CNS-metastasis are published and available, which should make authors able to mine candidates through genome-wide analysis. Among the 29 EOC cases with CNS-metastasis, both primary tumor and CNS-metastasis should be available for immunohistochemical-staining in 19 cases as they did for CD133-staining. If the authors successfully exhibited the up-regulated expression of candidate molecules/ TF in these pairs compared with those without CNS-metastasis, a future prospective study can be conducted for picking up cases which needs
close observation for CNS-metastasis.”

We have now included this point in the discussion and quoted the related references (p. 18): “Although we found that CD133 expression was a risk factor for the development of CNS metastases and that non-optimal cytoreduction and platinum resistance were risk factors for shorter time to the diagnosis of CNS metastases, identification of other potential markers such as transcriptional factors [1-4] and immune factors [5-7] in primary EOC to indicate future cisplatin-refractory CNS-metastasis in EOC patients was outside the scope of this study. A genome-wide transcription analysis may identify other candidate molecules that have different expression levels in EOC patients with and without CNS metastases.”

3. “CD133 expression in EOC was introduced as a “stem-ness” marker in Background part and Discussion part, but the interpretation of CD133+ population in the cell cluster of CNS-metastasis was not enough in the aspect of enrichment of cancer initiating cell. Previous reports demonstrated enrichment of cancer initiating cells in metastatic site or recurrence tumors. The result that all nine CD133+ cases exhibited CD133+ cluster in CNS-metastasis did match previous reports, but half cases did not exhibit CD133 in primary tumors. Was there no tumor initiating cells in such cases? Authors stated CD133- cells also had potency of tumor progression. Recent
reports emphasized the necessity to assess the stem-ness with several markers with such as CD44, ALDH, EpCAM as well as CD133. If authors did not employ CD133 as a simple marker to predict prognosis but a stem-ness marker, authors should deepen their interpretation of their result or conduct immunostaining of other markers for strengthening the analysis of stem-ness”

We agree with the Reviewer. The following sentence has now been included in the discussion to highlight this point (p. 17): “To better understand the role of a “stemness” marker of CD133 in the progression and metastasis of primary tumor, further studies are warranted preferably by analyzing a panel of potential “stemness” markers such as CD44, ALDH, EpCAM as well as CD133 in the future [8, 9].”

I trust that these changes address concerns of the reviewers. Please do not hesitate to contact me should you have any further questions.

Yours very sincerely,

Xiaoyan Xin, M.D., Ph.D.
References


