Author’s response to reviews

Title: Serum levels of soluble programmed death-ligand 1 (sPD-L1) in patients with primary central nervous system diffuse large B-cell lymphoma

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Serum levels of soluble programmed death-ligand 1 (sPD-L1) in patients with primary central nervous system diffuse large B-cell lymphoma

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BMC Cancer

Dear Sir,

Thank you very much for your valuable review of our manuscript. All comments were very helpful in revising and improving this report, and we did our best to respectfully follow your detailed advice. Heeding closely to the constructive comments suggested by the Editor and the reviewers, we have addressed and outlined each issue point-by-point in the attached texts below.

All of the authors have read and agreed with the revised manuscript. Again, thank you very much for considering our work for publication in this prestigious journal.
Best regards,

Seok Jin Kim, MD, PhD

Reviewer reports:

Herve Tilly, MD (Reviewer 1): The correlation of initial sPD-L1 serum levels and the outcome of DLBCL have been previously described. In this manuscript, Cho et al. study this parameter in a retrospective series of primary central nervous system lymphoma patients. The methods are well described and most of the results are interesting.

I have only minor comments:

1) The number of patients involved in each KM curve must be indicated

: We revised the KM curves as you recommended.

2) To state that there is a trend on the Figure 2D and E curves is exaggerated. The numbers in these groups are probably very small to allow any conclusion.

: As you mentioned, the number of patients in each group was too small to draw a conclusion. Thus, we revised those figures including figure 2D and E after we divided patients into two groups. Then, we revised those sentences in results (Results, page 13, line 5-11; Figure 2E, and F).

3) The results in Figure 3 expressing survival as a function of immunohistochemistry and sPD-L1 levels are very confusing and contradictory. This analysis could be deleted.

: We deleted figure 3A-C and the part about that in results as you pointed out.

4) The reference 25 could be replaced by the citation of the paper from the same authors in Leukemia 2017.

I am so sorry to say, I cannot find the paper you mentioned. It would be appreciated if you could let me know the full name of article published in leukemia 2017.

Outi Kuittinen, Ph.D (Reviewer 2): Comments:

1) Abstract raw 8: They should write PD-L1 protein instead of PD-L1

: We corrected it (Abstract, page 2, line 3).

2) Introduction should be shorter

: We revised the introduction according to your comments (Introduction, page 4, line 6-9; line 15-23; page 5, line 1-10).

3) Authors claim in the introduction that measuring sPD-L1 protein could substitute surgical biopsy. However, sPD-L1 can be elevated in healthy controls as well, and demonstrating elevated serum level cannot substitute diagnostic biopsy, even though, according to their results, it can give more precise prognostic information than PD-L1 immunohistochemistry.

: We revised the part of introduction according to your comments (Introduction, page 5, line 1-6).

4) Materials and methods: Authors do not give any description about the control cases, how they were selected, what was their age, sex distribution etc.

: We added the information about control cases in the method (Methods, page 8, line 10–13).

5) Authors have not performed EBV staining. Because EBV positivity is known to be associated with elevated PD-L1 expression in PCNSL, integrating this information to the data would be essential before drawing definitive conclusions. Also they should describe whether patients were immunocompetent or were there any patients with known immunosuppression.

: According to your comments, we have performed EBV ISH using the paraffin-embedded tumor tissue. Only one case was positive for EBV as well as PD-L1, however, the remaining patients were negative for EBV. We added the information about EBV ISH in method and results (Method, page 9, line 17-22; Results, page 13, line 11-13). In addition, there were no patients with known immunosuppression including patients taking immunosuppressive medications. Thus, all patients could be considered immunocompetent (Results, page 11, line 6-8).
6) Discussion: In results authors presented that tumor PD-L1 expression did not have statistically significant association with outcome. This is in line with previous publications. They found that sPD-L1 correlated with survival. However, in discussion they claim that sPD-L1 is a surrogate marker for tumor cell PD-L1 expression, which in turn, is prognostic for survival. I think it is not possible to draw this kind of conclusion from this study, even though there were some correlations between tissue PD-L1 expression and sPD-L1. There may be other interfering issues, why soluble protein is prognostic, but tumor expression is not. Authors should discuss about that.

: As you pointed out, we revised the part of discussion and added the comments on the discrepancy of sPD-L1 with PD-L1 in terms of the association with survival outcome. Based on our results, we could not provide clear explanation for the discrepancy. Thus, we mentioned the probability of other interfering issues related with the results (Discussion, page 16, line 10 – page 17, line 5).

In general, this is an interesting work studying the basic biology behind IOL therapies, which in future clearly have a role in the treatment of PCNSL as well. The study has a moderate sample size in such a rare disease. Authors have made a large number of serum cytokine analyzes in combination with tumor sample immunohistochemistry (IHC). IHC results are analyzed separately in different cell types to gain proper information. However, I would miss some more detailed data concerning patients’ immunology. Also, I would not fully agree with the some of the conclusion drawn from the study.

: I agree with your opinion, thus, we provided more detailed data and revise some points of the conclusion drawn from our study.

Harald Holte (Reviewer 3):

The Abstract, Introduction, Methods and Results sections are fine. In the discussion I would suggest to add that

-in a clinical multicentre setting, the small deviation of the SPD-L1 Levels in the cohort compared to normals will be problematic

-the results should be validated in a separate cohort.

: As you pointed out, the deviation of sPD-L1 levels of patients compared to normal controls remains as a problematic issue, and the validation of our results using a separate cohort should be considered. However, this disease entity is very rare, so, it is very difficult to find an independent prospective cohort together with serum samples. Indeed, our cohort study is only one prospective study for lymphoma in Korea. Thus, we could not validate our results with another cohort sample. However, we will do a subsequent study confirming our current results with our next cohort study samples. We mentioned this limitation in the section of discussion (Discussion, page 17, line 3-5; Conclusions, page 17, line 22-23).
Minors:

-p5, lines 24-25: what is meant by clinical manipulation of PD-L1 Expression?

: The meaning of that sentence was as follows. ‘As PD-L1 could be expressed in non-malignant cells, it might be often difficult to determine PD-L1-positive cases from patients with PCNSL’. However, we thought that sentence could be omitted, so we deleted it, and re-wrote the part (Introduction, page 5, line 17-23).

-p6, line 24-25 should read: In Our prospective cohort studies, we collected serum samples and...

: We corrected the sentence according to your comments (Methods, page 6, line 5-6).