Author's response to reviews

Title: Ki-67 is a valuable prognostic predictor of lymphoma but its utility varies in lymphoma subtypes: evidence from a systematic meta-analysis

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Author's response to reviews:

Dear Editor,

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers’ comments are listed below this letter.

We hope that the revised version of the manuscript is now acceptable for publication in your journal.

I look forward to hearing from you soon.

With best wishes,
Yours sincerely,
Liansheng Huang

We would like to express our sincere thanks to the reviewers for the constructive and positive comments.

Replies to Reviewer 1

Specific Comments

Comment 1#I am not sure, if cut-off positivity was somehow taken account in this analysis. I strongly recommend to emphasize or better comment following facts:

- different cut-off values for Ki-67 index were identified in many retrospective studies, ranging from 10 to 50% (also even if immunohistochemistry used),
- the application of the Ki-67 index in experimental as well as in routine management is limited by relatively high inter-observer variability.

Answer# The different cut-off value for Ki-67 index is an important factor that
should be taken account in this analysis. As recommended by reviewer, we have added the comments into the discussion part of manuscript in page 15 line 9-18.

Comment 2
Conclusion concerning mantle cell lymphoma and loss of prognostic significance of Ki-67 in rituximab treated patients (based totally on 2 studies only!) is a little bit strange and must be precisely commented. For example, the most recent analysis of Salek and colleagues (Leuk Lymph 2013) which included 144 rituximab-treated patients, revealed that patients with the proliferation rate 30% (Ki-67) and more have significantly higher relative risk of death and shorter overall survival (median OS 22.6 vs 76.4 months, p<0.001).

Answer
It is definitely correct that the more studies assessed, the more convincing the results. To include more studies, we recently searched for updated studies on the PubMed and Web of Science databases, and identified a study conducted by Salek et al. Therefore, we performed a meta-analysis again on these identified studies, and found that in MCL, high Ki-67 expression was not only related to a poor OS with rituximab (HR=1.981, 95%CI: 1.099-3.569, p=0.023), but also correlated with survival outcome without following rituximab treatment (HR=3.123, 95%CI: 2.049-4.76, p=0.000).

Comment 3: It is necessary to be aware of limitation of rituximab influence itself on prognosis, because significant global changes in treatment strategy (not only rituximab addition) were made since year 2000, which are mirrored in general improvement of overall survival in MCL and DLBCL patients. This fact can also influence the relationship rituximab and Ki-67. Please, comment and explain more in detail the treatment regimens at least main subgroups like high-dose chemotherapy, antracyclins, or more intensive treatment like MaxiCHOP given with or without rituximab subgroups. The same is valid for DLBCL. Please, the sentence…” Ki-67 expression has prognostic value in DLBCL only following rituximab treatment, while in MCL…”", I think that this interpretation must be corrected....

Answer
There is no doubt that further subgroup analysis in rituximab subgroups according to treatment regimens like high-dose chemotherapy, antracyclins, or more intensive treatment could reduce the influence of confound factor. Nevertheless, because the included studies didn’t provide relevant data, we couldn’t calculate the value of HR according to more detailed treatment regimens. Therefore, owing to the existence of some confound factors, like different population, study method, treatment regimens and so on, heterogeneity is inevitable in meta-analysis. But in our meta-analysis, both the result of subgroup analysis and sensitivity analysis suggested that the outcome of our study is stable and reliable. As recommended by reviewer, we have replaced the sentence with “in DLBCL, Ki-67 expression has prognostic value following rituximab treatment but the prognostic value diminishes without following rituximab treatment, while in MCL, Ki-67 expression was a negative prognostic index whether or not following rituximab treatment.” in our manuscript. Amendments are highlighted in red.
Comment 4: It is necessary to mention, that known negative prognostic factor naturally influence the choice of therapy in these patients, and so, the positive impact of adjusted treatment strategy can paradoxically decrease prognostic significance of such index.

Answer#The recommendation of the reviewer is reasonable and sensible. However, in our manuscript, we evaluated the prognostic significance of Ki-67 expression in lymphoma and in DLBCL and MCL after the introduction of a CD-20 monoclonal antibody, rituximab. We identified that ki-67 is indeed a negative prognostic factor in lymphoma. Nevertheless, the adjusted treatment strategy (administration of rituximab) is related to the expression of CD20 rather than the expression of Ki-67. Therefore, ki-67 is a valuable negative prognostic factor in lymphoma but it is not correlated with using rituximab.

Comment 5: Please, comment and interpret your results very carefully also in abstract! Also, in the abstract please specify, which clinical—pathological features of lymphoma have no correlation with Ki-67.

Answer#We have revised the results of the abstract and specified the clinical—pathological features of lymphoma according to the recommendation of reviewer.

Comment 6:
1) Some english sentences seem to be a little strange, please review english
2) Please, give better titles, comments and footnotes of Figures and Tables

Answer#This manuscript has been edited and proofread by Elsevier Limited and we have reviewed and revised our manuscript again. The titles, comments and footnotes of Figures and Tables have also been revised. The amendments are highlighted in red in the revised manuscript.

Replies to Reviewer 2
Specific Comments

Comment 1#In their abstract the authors suggest that they analyzed the impact of Ki-67 expression on survival in various subtypes of lymphoma and after the introduction of rituximab. In the later parts of the manuscript it becomes clear (as expected by the fact that rituximab can only be used for therapy in the case of CD20-expressing B-cell lymphomas) that they could analyze the rituximab effect only in diffuse large B cell lymphomas and mantle cell lymphomas- and of course not in Hodgkin lymphoma and NK/T cell lymphoma which do not express CD20. This should be mentioned in the abstract, which should be modified accordingly.

Answer#The description in our abstract may be not clear enough. Accordingly, we have revised it both in our abstract and in other parts of our manuscript.

Comment 2#The authors use the terms of „Ki-67 overexpression“ as well as the presence of Ki-67- positive“ and „Ki-67-negative patients“. As the authors correctly state Ki-67 is expressed in all phases of the cell cycle. It is therefore...
hard to understand that there might be Ki-67 "overexpressing“ cells and malignant tumors like the lymphomas studied here, which are Ki-67 negative. As Ki-67 is constantly expressed in normal and in neoplastic cells it would be more fitting to use the terms of high and low Ki-67 index, or high/low proliferative activity respectively.

Answer#As recommended by reviewer, we have replaced the terms such as “Ki-67 overexpression”, “the presence of Ki-67- positive” and “Ki-67-negative patients” with “high and low Ki-67 index” in our manuscript. Amendments are highlighted in red.

Comment 3#Another term that should be amended is the „subgroup of malignant lymphoma“. Actually the WHO lymphoma classification does not classify lymphomas into subgroups but into disease entities.

Answer#We have amended the term in our manuscript, and a new paper (Blood 1997, 89(11):3909-3918 ) from the Non-Hodgkin's Lymphoma classification project was quoted to demonstrate the classification of non-Hodgkin's lymphoma.

Comment 4#The authors state that they included in their study only publications dealing with the immunohistochemical detection of Ki-67. There are however some important informations missing :
- which Ki-67 antibody was used in the various studies?
- how was the percentage of Ki-67 positive cells evaluated (estimated or by image analysis)
- did the studies use tissue microarrays or whole tissue sections?

Answer#As recommended by reviewer, we have added the source of antibody, the method of evaluation, and the source of sample into the Table 1.

Comment 5#Another fact that is not sufficiently clarified for the readership is how the authors could extract reliable information from studies dealing with biologically different patient groups suffering from the same lymphoma entity. For example the two Hodgkin lymphoma studies selected are dealing either with only young patients (Barros et al.) or only with advanced disease stage (Morente et al.). This heterogeneity is also obvious in the diffuse large B-cell lymphoma group with studies including only young patients with good prognosis (Pfreundschuh et al.) and others with aggressive lymphoma and advanced stages (Jerkeman et al.)

Answer#We have to admit that heterogeneity resulted from different patient groups is inevitable in meta-analysis. However, firstly, we have used random effects models to analyze the data, which could reduce the influence of great heterogeneity on the reliability of results. Secondly, in our meta-analysis, subgroup analysis of OS was performed according to various strata and the results revealed that the negative association between high Ki-67 expression and
OS was present across all strata. Finally, sensitivity analysis was a tool detecting the impact of anyone included study on the overall result. We also performed sensitivity analysis and the result of sensitivity analysis was showed in Table 3. We found that the pooled HR ranged from 1.67 (95% CI: 1.4 – 1.99) to 1.87 (95% CI: 1.49 – 2.35) after excluding the study of Chung et al and the study of Determann et al respectively. It indicated that the relationship between Ki-67 expression and prognosis of lymphoma was not changed after excluding anyone study. Above all, we have decreased the effect of heterogeneity on the result greatly through the methods described above.