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Editor-in-Chief

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RE: ESPS Manuscript NO: 13355

Dear Editor-in Chief

Thank you for the careful review of our manuscript entitled “Coexpression of MYC and BCL-2 predicts prognosis in primary gastrointestinal diffuse large B-cell lymphoma” and the opportunity for revision. We appreciate the encouraging comments provided by reviewers. We thank the reviewers for their constructive critiques of our manuscript and for their excellent suggestions. The following is a detailed response to each comment and we believe that by addressing their concerns we have added clarity to our manuscript.

General comments:

- 1. What was the difference in significance of examining MYC/BCL-2 expression in PGI-DLBCL from the examination in DLBCL? In DLBCL, Johnson et al. (J ClinOncol, 2012) had published the excellent study.**

Response: This is a very good point. In the article mentioned above (J ClinOncol 30:3460–3468, 2012), Johnson and colleagues report a study that used c-Myc antibody in combination with an antibody for BCL2 in a training cohort of 167 and a validation set of another 140 patients with DLBCL. They found MYC translocations, high MYC mRNA, and MYC protein expression in 11%, 11%, and 33% of the samples, respectively. MYC protein expression was associated with an inferior progression-free and overall survival only when BCL2 protein was coexpressed. However, most of DLBCL cases in this study are nodal DLBCL (Table 1 of the paper). PGI-DLBCL in this series are relatively small and the incidence and significance of MYC or/BCL2 expression was not mentioned in this study and have not been reported in the literature. Given the specialized location of the GI tract and GI lymphoma association

with infections such as H pylori infection, celiac disease, inflammatory bowel disease and autoimmune diseases, we considered PGI-DLBCL as a distinct since their evaluation, diagnosis, management and prognosis are different from DLBCL of lymph node origin (nodal). Thus, our study is performed to focus on primary gastrointestinal DLBCL, and we observed the rate of concurrent expression (MYC positive/BCL2 positive) is higher in PGI-DLBCL than all of DLBCL (30% VS 21%), suggesting that concurrent expression of MYC and BCL2 is more frequently in PGI-DLBCL. We have revised the introduction of this manuscript to provide the rationale for this study and the revision highlighted in yellow.

2. Discussion section would be too long. Authors could reduce the repetition.

Response: We agree that many portions of our discussion were redundant. As suggested, we have revised the discussion and made it concise and more focused.

Minor comments:

- 1. Materials and Methods, Patients: Authors would better demonstrate the expression status of CD20, CD10 and possibly CD5 in lymphoma cells of PGI-DLBCL patients. Also the chromosomal abnormality data would have significance.**

Response: In fact, all of our cases, the immunohistochemistry for the expression of CD5, CD10 and CD20 is performed routinely in all of our cases as a diagnostic panel. We have analyzed the expression of CD5, CD10 and CD20 as well as CD3, but revealed that there is no statistically significance between positive and negative results of any these CD markers. As suggested, we have added these data to the table 2 showing phenotypical characteristics of the cases. Also, we agree that chromosomal abnormality data would have significance since there is consensus that MYC translocations confer a worse prognosis in patients with DLBCL treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), both in combination with and without rituximab (Savage KJ et al, Blood 114:3533–3537, 2009, Barrans S et al, J Clin Oncol 28:3360–3365, 2010). However, the chromosomal data were not included in this study because 1) cytogenetic and FISH were not performed

in most of our cases (due to cost and technical limitation) 2) instead we intended to focus on MYC and BCL2 protein expression study by using recently developed and commercially available monoclonal antibody. The antibody targets the N-terminus of the MYC protein, has been shown to predict MYC rearrangements and has been validated for use FFPE tissues. We believe that molecular confirmation of the immunohistochemistry for MYC and BCL2 are more practical, relevant and closely representative. This is based on: in addition to translocations, MYC can also be deregulated by amplifications, mutations, or by microRNA-dependent mechanisms. Although MYC translocations can be detected by karyotype and fluorescence in situ hybridization (FISH), FISH fails to detect MYC deregulation caused by mechanisms other than translocation. This suggests that mechanisms other than gene rearrangements are responsible for elevated protein expression in a considerable proportion of DLBCL cases. Johnson et al (JCO, 2012) indeed found MYC translocations and MYC protein expression in 11%, and 33% of the samples, respectively supporting the above notion. We have integrated these in the manuscript highlighted in yellow.

2. Materials and Methods, Patients: Normal control samples were taken from lymphoid tissue. Are they lymph nodes? Tonsils? Lymph follicles in the gastric mucosa?

Response: We apologize that our description about these experiments was not clear and confusing. Normal control samples are reactive lymph nodes and have added in Materials and Methods.

3. Materials and Methods, Immunohistochemistry: Please indicate the reason why the authors selected 30% as the standard for positive.

Response: This is an excellent question. Cut-off value for MYC expression has been varies in different studies from different medical centers. For instance, Johnson et al (JCO, 2012) used 40% as cut-off value for low and high Myc groups of de novo DLBCLs while Kluk et al chose 50% of positive Myc positive cells as the cut-off point for de novo and relapsed DLBCLs (PLoS One. 2012;7(4):e33813). We established a cut-off value of 30% for PGI DLBCLs in this study based on that 30% cut-off point is

best value for dichotomizing expression of MYC protein expression and shows best correlation with clinical outcomes of our patients. However, It should be noted that our cohort of patients with outcome data is small (60 patients), and that these results will need to be validated in additional patient cohorts and across multiple institutions.

Again, thank you and the reviewers for comments, efforts and consideration.

Sincerely,

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