Reviewer's report

Title: Activation of nucleophosmin-anaplastic lymphoma kinase/ protein kinase B /mammalian target of rapamycin signaling pathway in anaplastic large cell lymphoma and its correlation with the clinicopathologic variables

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Reviewer: Lukas Kenner

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The manuscript Ju Gao et al. „Activation of nucleophosmin-anaplastic lymphoma kinase/ protein kinase B/mammalian target of rapamycin signaling pathway in anaplastic large cell lymphoma and its correlation with the clinicopathologic variables“ show that in ALK-negative samples are overrepresented in p-AKT negative samples, suggesting an relative higher activation of the AKT pathway in the patients with ALK translocations.

This is an interesting manuscript, however a number of the data shown were already published elsewhere. In addition the study completely relies on IHC staining of patient samples and do not include any more mechanistic in vivo data to support the authors hypotheses, which would be important to support the translational relevance of this manuscript for possible future diagnostic and therapeutic approaches.

In addition, the authors did not verify their data with state of the art quantification techniques.

Major:

As expected, the mTOR pathway is mostly higher activated in patients bearing the ALK translocation. However localization of the NPM-ALK fusion protein seems to be of no consequence for prognosis and pathway activation in ALCL.

Therefore the data suggest that the AKT-mTOR axis is over-activated in 70-80% ALK+ ALCL cases. This suggests that mTOR as well as the AKT pathway are crucial for tumor growth in ALCL, ALK+ and probably less so in ALCL, ALK-, however this is not proven in the current version of the manuscript.

1. the authors should use ALCL cell lines (Karpas 299, SR786, SU-DHL1, Mac1, Mac2a) with and without the ALK translocation and treat them with different AKT and mTOR inhibitors, such as NVP-BEZ235, which is a dual PI3K and mTOR inhibitor.

This would give direct proof about the importance of the described pathways for ALCL tumor growth in ALK+ and ALK- tumors.

Since the AKT1 kinase has two homologs AKT2 and AKT3, it would be interesting to know the respective contributions of these homologs to the
AKT/mTOR pathway activation. This should be feasible since there are inhibitors available that bear a certain specificity for the different AKT homologs.

Alternatively, the authors could do independent knockdown of AKT1/2/3 using antisense or small hairpin or siRNA techniques in the cell lines mentioned above and study activity of the AKT/mTOR pathway by 4EBP1 and S6K phosphorylation to dissect the differential importance of the homologs.

2. To demonstrate the in vivo significance of their findings, authors should xenograft the above mentioned cell lines into mice and demonstrate the growth pattern and target gene expression levels after mTOR and AKT1/2/3 inhibition.

3. State of the art quantification software (such as Histoquest or else) should be applied to quantify protein expression levels and percentage of tumor/stromal cells affected from the tumor samples.

Minor:
1. On page 8 a table VI is mentioned, which should be shown.
2. Please carefully correct several typos.

**Level of interest:** An article whose findings are important to those with closely related research interests

**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