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

Version: 2 Date: 28 March 2013

Reviewer: Emmy D.G. Fleuren

Reviewer's report:

Thank you for the opportunity to review this manuscript. This is an interesting study linking ALK expression to activated downstream targets (AKT/mTOR) in ALCL. The authors also investigated possible correlations between expression of these proteins and patient outcome. There are however several concerns that need to be addressed prior to publication that will improve the quality of this work.

Major Compulsory Revisions:

1. Language corrections are necessary throughout the whole manuscript before it can be considered for publication. I have indicated the general problems and some (not all) specific examples below, but would highly recommend it to be fully edited by a native speaker. Without extensive editing, I’m afraid that it is hard for readers to go through the paper and fully understand the scientific content.

General problems:
- The words ‘the’ and/or ‘a’ are often missing
- Incorrect use of words
- Punctuation often incorrect

Some specific examples:
- Abstract; background: Activation of AKT/mTOR pathway -> Activation of the AKT/mTOR pathway ór activation of AKT/mTOR
- Abstract; background: ‘from clinical perspective’ -> from a clinical perspective
- Abstract; background: associated -> # correlated
- Abstract; background: prognostic value -> # the prognostic value
- Background; 1st paragraph: accounts for up to 30% to 40% -># accounts for up to 40% ór accounts for 30% to 40%
- Background; 2nd paragraph: later -># latter (also discussion; 2nd paragraph)
- Background; 3rd paragraph: While -># Because the…, indicating -> # this indicates ór The…, indicating
- Background; final paragraph: In present study -> # in the present study
- Discussion; 1st paragraph: much in consistent to -># correlated well; literatures -> # literature
- Discussion; 2nd paragraph: .. ->#.
- Discussion; 2nd paragraph: whole ALCL patients -># all ALCL patients

2. Title: The authors state that the NPM-ALK/AKT/mTOR pathway is activated in ALCL. Although this is true for the AKT/mTOR pathway by showing p-AKT en p-mTOR expression in the ALCL cohort, it is not certain whether this is solely caused by (NMP/)ALK activation. Although the authors have indeed linked ALK expression to p-mTOR and p-AKT expression, they have not considered p-ALK levels. Since AKT and mTOR signal downstream of numerous other (receptor)proteins, these may also contribute and significantly correlate to p-AKT/p-mTOR signaling. This concern should at least be addressed in the discussion section.

3. Methods; immunohistochemical studies: In this section, the authors mention the use of two ALK antibodies (ALK11 and ALK-1). It is however unclear why they use/mention two different ALK antibodies, while in the results section only results of the ALK-1 antibody are given (Figure 1). Does this mean that the ALK-1 antibody was used to stain the 103 cases? What was then the purpose of the ALK11 antibody? In addition, dilutions of the ALK antibodies are missing in the methods section, while for all other antibodies dilutions are given.

4. Methods; immunohistochemical studies: it is unclear how the immunohistochemical stainings were scored/interpreted. It is important to clarify this, since all study results are based on these stainings and IHC results are subject to interpretation. Are sections scored based on staining intensity levels? Was the number of positive cells included / was there a cut-off for % of positive cells? When was a sample considered to be positive (for instance, if very weak staining was observed, was this considered to be positive or negative?)? Who/how many persons were involved in interpreting the stainings? It is especially important to address these issues because the authors subsequently divide the cohort in “+ or –” expression for all the proteins they investigated, but IHC results are often not that black-and-white. In addition, representative pictures of both + and – scored slides for at least ALK, p-AKT and p-mTOR proteins should be included, and preferably also for p-4E-BP1 and p-p70S6K1.

5. Results; The prognostic significance of the expression of ALK etc. section; second paragraph: table VI is missing. In addition, VI should be 6 (in line with other tables).

Minor Essential Revisions:
1. Abstract; methods: ATK/mTOR -># AKT/mTOR
2. Abstract; methods; “Expression of ALK and AKT/mTOR signaling phosphoproteins..” This sentence implies that the authors also looked at phosphorylated ALK protein, while only for AKT/mTOR phosoantibodies were
used. The sentence should be rewritten to avoid misinterpretation by readers.

3. Methods; study design: The 103 cases consist of 62 ALK+ALCL and 51 ALK-ALCL cases (gives total of 113 patients instead of 103)? Probably a mistake; adding up the ALK- cases in table 1 gives a total of 41 ALK- patients.

4. Results: The prognostic...and p-p70S6K1; 1st paragraph: ALK, p-AKT, and p-mTOR signaling phosphoproteins -># ALK, p-AKT, and p-mTOR signaling proteins (p-AKT/p-mTOR already indicates phosphorylation)

5. Discussion: Information given in the first paragraph seems more appropriate for the introduction.

6. Discussion; 1st paragraph: 15 NPM-ALK variant fusion genes -> # 15 ALK-variant fusion genes?

7. Discussion; 3rd paragraph: kinsase -># kinase

8. Discussion; 3rd paragraph: AKT+/mTOR pathway -># AKT/mTOR pathway?

9. Discussion; 4th paragraph: prognositic -># prognostic

10. Figure 1 looks somewhat chaotic and confusing because many different magnifications are used. It feels more appropriate to show all pictures with the same, not too high, magnification (e.g. 200x), especially because remarks are made concerning the size of cells (Figure 1a shows large tumor cells and 1b small tumor cells, which is not surprising since 1a is 400x and figure 1b 200x). If the authors would like to highlight a specific staining pattern, I would suggest to include a higher inset magnification in addition to the lower magnification. The pictures are also a bit blurry; I would suggest to take clearer pictures which will make the manuscript look more professional.

11. Figure 1; end of legend: ‘No negative control staining was included’. This sentence suggests that no negative control stainings were performed at all, while in the methods section it is mentioned that controls were performed by omitting the primary antibody. If negative controls were used to verify the reliability of all antibodies used, and were indeed all negative, this sentence can be excluded from the figure legend to avoid misinterpretation.

Discretionary Revisions

1. Title: The authors state that the NPM-ALK/AKT/mTOR pathway is activated in ALCL. This however does not accurately convey what has been found. The authors have indeed found a correlation between ALK expression in general and p-AKT/p-mTOR, but they also state that neither p-AKT nor p-mTOR was related to ALK subcellular expression patterns. Since these subcellular expression patterns distinguish NPM-ALK from other ALK-variants, they conclude that ‘no matter what the ALK fusions they are, they can activate AKT/mTOR pathway’ (language corrections also necessary in this sentence). The title suggests that specifically the NPM-ALK fusion correlates to AKT/mTOR pathway activation, but
it could actually be any fusion. I would therefore suggest to delete the word ‘nucleophosmin’ from the title.

2. Figure 1; legend: for all IHC stainings, ‘EnVision staining’ is mentioned, but this system is not specifically mentioned in the methods section. It would be logical to mention EnVision already in the Material and Methods section.

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

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests