Reviewer's report

Title: Clinicopathologic features and prognostic implications of NOK/STYK1 protein expression in non-small cell lung cancer

Version: 2 Date: 21 November 2013

Reviewer: Radostina Cherneva

Reviewer's report:

1. The article “Clinicopathological features and prognostic implications of NOK/STYK1 protein expression in non-small cell lung cancer” by Peng et al, treats one of the major issues in up-to-day oncology – the establishment and validation of biomarkers, that could have potential diagnostic, prognostic or predictive value in clinical practice. The study is presented by a clear-cut aim – the determination of NOK protein in NSCLC biology, as well as, its prognostic value. The design of the study is clear and is presented very concisely to the reader to make it easily apprehensible. Moreover it is intriguing as far as it presents data, regarding the expression of a tyrosine – kinase receptor protein, whose role in NSCLC tumorigenesis is still unknown.

2. The method of the study - immunohistochemistry is well described. The antigen retrieval procedures for Ki-67 are kept, as well as the major steps for NOK performance. I have some remarks and comments regarding the method in general. In is now clear that when a marker should be validated into clinical practice, immunohistochemistry is not the first, but the last step in validation. It usually starts with proteomics – gene expression and real-PCR studies and finally comes to Western blotting and immunohistochemistry. As we have no data about the general behaviour of NOK in NSCLC, except for the gene expression study by Amachika et al, I think immunochemistry is not the best way of making any statements.

3. A strong point in the study is that the data is very clearly presented in the text, as well as, in the tables attached.

4. The article adheres to the general standards for data reporting and deposition.

5. The discussion and conclusions are well balanced. I think that the limitations of the study should be mentioned here. This would somehow compensate for the drawbacks of the study and the reader won’t be misled by the final results. I think the study has four major limitations.

   The first drawback is the small number of patients; the second is the follow-up period – it is too short and limits the Kaplan Meier; the third is the method as I already mentioned. The choice of the cut-off points for NOK and Ki 67 are not argumentably presented.

6. I do not think that any limitations are mentioned at all. I recommend that they should be: 1) the method is not the best one for a biomarker validation; 2) the follow up period is the other major drawback – median survival is not reached in
many of the groups, presented.

7. The article mentions two studies that are connected with NOK role in tumor biology. The one is that by Liu et al, A novel protein tyrosine kinase NOK that shares homology with platelet-derived growth factor/fibroblast growth factor receptors induces tumorigenesis and metastasis in nude mice. Cancer Res, 2004 and the other one is that by Amachika et al. The authors use mainly the results from Liu et al, to discuss and interpret their results. The study of Amachika is only briefly depicted and cited, as it uses a totally different method for investigation. As there are only two studies about NOK role in NSCLC (Amachika et al, and Zing et al, ) I think that the authors are doing well in acquainting the reader with the basic findings of NOK in NSCLC cancerogenesis.

8. The title and abstract clearly convey the aim of the study, what is done, what are the results and conclusions.

9. The writing and style are acceptable.

Major Compulsory Revisions

1) The distribution of the tumors regarding some of their clinicopathological features (grade, tumor stage and lymph node metastases) is uneven, which makes the statistics unreliable.

2) The second drawback is the follow-up period – it is too short and limits the Kaplan-Meier in retrieving the median survival period. Mean survival period is mostly mentioned and this, we know, is not as a reliable measure for survival and thus for prognosis. Moreover, if we look the data in detail, we shall see that the median survival is not achieved in any of the NOK negative tumors, which makes the prognostic value of the marker doubtful. I am not sure that the general reader knows exactly what mean and median survival is, this may cause confusion and may deter the comprehensibility of the results.

3) The method as I already mentioned is not an appropriate one.

4) Another drawback, which is very important, is the semi-quantitative way of interpreting the results of the immunochemical study of NOK - it is very subjective.

The reason for the choice of these cut-off points is not well described. As this is the first immunostudy of NOK the choice of the cut-off points is of major importance for the interpretation of the results. The ROC analysis that is mentioned deserves a more detailed description.

5) A fifth limitation is the cut-off point of Ki 67- 31%. In NSCLC, in contrast to breast cancer, there is not a consensus about the cut-off of Ki-67 for the division of tumors up to their proliferation rate. If a cut-point should be used for this study, it should be reconsidered up to the mitotic index and based on it. It ought not be the median or average Ki-67 LI, that should be applied as a cut-off. This again is very important for the future interpretation from the viewpoint of NOK expression.

Minor Revisions
Please note that the adjacent tumor tissue is rarely normal – it always has some dysplasia. It should be adjacent non-cancerous tissue instead.

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

**Quality of written English:** Acceptable

**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'