ANSWERING REVIEWERS

March 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8734-Review.doc).

Title: C4.4A as a biomarker in pulmonary adenocarcinoma and squamous cell carcinoma

Author: Benedikte Jacobsen, Mette Camilla Kriegbaum, Eric Santoni-Rugiu, Michael Ploug

Name of Journal: *World Journal of Clinical Oncology*

ESPS Manuscript NO: 8734

We are grateful for the efficient and constructive review process of our manuscript, which has been improved according to the suggestions of the five reviewers, as detailed point by point in the following and as highlighted with track-changes in the revised version.

Reviewer 1

1. What experimental methods dose this paper use to obtain the resulting picture

The format for a review does not include a Materials and Methods section, which explains why this has not been the focus of the present topic highlight. The reviewer is therefore referred to the original articles, where a detailed description of experimental methods is provided (*e.g.* Jacobsen et al, Int J Cancer 2012, 130: 2734-2739 and Jacobsen et al, JTO 2013, 8: 152-160). That being said, there is throughout the manuscript and in the figure legends considerable reference to the methods used (*e.g.* semi-quantitative immunohistochemical protocol using a polyclonal C4.4A antibody, Kaplan-Meier analysis, multivariate overall survival analysis by the Cox proportional hazards model), which should answer the concern of the reviewer.

2. This paper only made a review for squamous cell carcinoma and adenocarcinoma which is not entirely representative of non-small cell lung cancer, however the title of the paper is about non-small cell lung cancer biomarkers

The title has been changed from "*The Ly6/uPAR protein C4.4A as a biomarker in non-small cell lung cancer*" to "*C4.4A as a biomarker in pulmonary adenocarcinoma and squamous cell carcinoma*" to reflect the fact that, as correctly observed by the reviewer, only the histologic subtypes of adenocarcinoma and squamous cell carcinoma have been considered in the text.

3. References required uniform format

We fully agree with this comment and have now updated the format of the references according to the requirements of the *World Journal of Clinical Oncology*. In the few cases where we have been unable to locate the DOI, we have linked to the most recent version of the given article available on the internet.

4. What advantages dose it has compared with other tumor markers, and what is the future research directions.

The reviewer points to a very crucial and intriguing question, to which we unfortunately do not have the full answer yet. As mentioned in the manuscript, this would first require a retrospective test for

superiority of C4.4A to current prognostic factors *e.g.* by using material from previously conducted large clinical trials, and subsequent validation in a prospective, randomized trial. Our multivariate analysis of overall survival shows, however, that C4.4A is a significant independent prognostic factor, meaning that it yields information additional to that given by stage, which is also a significant prognostic factor and currently used in the clinic. Despite a correlation to solid AC, C4.4A expression is furthermore a stronger prognostic factor than solid growth. As discussed in the manuscript, C4.4A also has a potential role as a marker of early precursor lesions in the progression to SCC and AC. The usefulness of C4.4A as a clinical marker is thus worth further, more resource-demanding, and possibly multicenter-based studies. For this reason, we have not specifically addressed the future research directions for delineating the role of C4.4A in non-small cell lung cancer in a separate paragraph, but rather as suggestions throughout the manuscript, including crossing the C4.4A-deficient mouse with the KRAS/LKB1 lung cancer model, investigating the putative inverse correlation between C4.4A and LBK1 and testing the potential of C4.4A as a predictive biomarker for treatment targeting the LKB1 pathway.

Reviewer 2

C4.4A is a cell membrane protein, roles as a potential biomarker in in non-small cell lung cancer. In this paper, the authors give a review of the role of C4.4A in NSCLC, which is valuable and attracts great interests for the researchers in this field.

We thank for the reviewer's acknowledgement of the relevance of C4.4A as a potential marker in non-small cell lung cancer.

Reviewer 3

The review "The Ly6/uPAR protein C4.4A as a biomarker in non-small cell lung cancer" by Jacobsen et al. describes the prognostic and predictive validity of C4.4A in non-small cell lung cancer. Being expressed in suprabasal layers of stratified squamous epithelia, it is absent from healthy bronchial and alveolar tissue, but present at early stages of lung cancer. Surprisingly, it is also expressed in a fraction of pulmonary adenocarcinoma, which correlates with poor survival and a solid growth pattern. Additionally, there appears to be an inverse relationship between C4.4A and the tumor suppressor LKB1. The authors first introduce lung cancer, new therapeutic concepts based on TKI and point out that new biomarkers are also relevant for improving prognosis, one of these markers being C4.4A. The authors introduce C4.4A expression in health and disease and then focus on C4.4A in pulmonary squamous cell carcinoma. The authors explain that C4.4A expression correlates with the differentiation status and not malignant potential, but might be involved in transdifferentiation. In the following chapter the authors are concerned about rare and weak expression in AC. They describe that C4.4A expression is tightly correlated with the solid growth pattern and is a stronger prognostic factor than solid growth. They further speculate that C4.4A-positive AC are of the squamoid type and on the inverse correlation with the tumor suppressor LKB1. This is a very thoroughly and well written review on C4.4A. I have only one concern that the second part is too lengthy such that the potential reader gets tired and looses interest. The review would greatly profit, when the authors try to significantly shorten the second part.

We greatly appreciate this thorough and once again positive assessment of our review. As suggested by the reviewer, we have shortened the second part of the manuscript substantially and believe that this has yielded a tighter discussion of the role of C4.4A in pulmonary carcinomas. The part dealing with C4.4A in AC is still considerably longer than the part concerning SCC, and this pertains to the clinical relevance of C4.4A in AC as compared to SCC, where very distinct aspects have to be covered:

1) The stepwise development from AAH to AC
2) Correlation to prognosis
3) Growth pattern analysis
4) Pathway regulation

Reviewer 4

This article is well designed and brings honest, professional and interesting information on molecular biomarkers of lung cancer. It will be useful for molecular biologists and oncologists who in their practice encounter questions of cancer biomarkers. I recommend this article for publication in World Journal of Clinical Oncology.

We greatly value that the reviewer considers our article to be of high quality and of significant interest to the readers of the *World Journal of Clinical Oncology*.

Reviewer 5

The authors describe the knowledge on Ly6/uPAR protein C4.4A and its role as biomarker in non-small cell lung cancer. The paper is well written and underlines the potential usefulness of C4.4A as marker in AC and in SCC patients. Moreover the possible role of C4.4A as an early diagnostic tumor marker in precursor lesions is discussed too. To further clarify for the readers the potential role of C4.4A in NSCLC as a marker, it should be reported a table resuming the finding about C4.4A expression in lung tissues and its relation with the progression of the disease.

As proposed by the reviewer, a table summarizing the expression of C4.4A in premalignant and malignant lesions both in the progression to AC and SCC has now been included, which has improved the reader-friendliness of the review. Results from the multivariate analysis of overall survival by the Cox proportional hazards model have likewise been incorporated in the table, which clearly shows the prognostic value in AC, but not in SCC, and further underlines the different role of C4.4A in these two histologic lung cancer subtypes.

All in all, we feel confident that we have answered the concerns raised by the reviewers, and we think that this revision has indeed resulted in a more focused discussion and improved the clarity of the manuscript. Based on the consistent positive feedback, we believe that our topic highlight represents an important contribution to the *World Journal of Clinical Oncology*. We very much look forward to receiving your comments on our revised review and thank you for taking our manuscript into consideration for publication in the *World Journal of Clinical Oncology*.

Sincerely yours,

Benedikte Jacobsen, PhD
The Finsen Laboratory, Rigshospitalet
Copenhagen Biocenter
Ole Maaloes Vej 5,
2200 Copenhagen N, Denmark
Fax: +45-35453797
E-mail: bjacobsen@finsenlab.dk

Michael Ploug, dr.scient
The Finsen Laboratory, Rigshospitalet
Copenhagen Biocenter
Ole Maaloes Vej 5,
2200 Copenhagen N, Denmark
Fax: +45-35453797
E-mail: m-ploug@finsenlab.dk