Author's response to reviews

Title: EGFR and HER2 expression in primary cervical cancers and corresponding lymph node metastases: Implications for targeted radiotherapy

Authors:

Li Shen (lishenhz@yahoo.com)
Yongjie Shui (yjshuihz@yahoo.com)
Xiajia Wang (xiwangzi@tom.com)
Liming Sheng (lmshenghz@yahoo.com)
Zhengyan Yang (zyyangzi@yahoo.com)
Danfeng Xue (dfxuezj@yahoo.com)
Qichun Wei (Qichun_Wei@zju.edu.cn)

Version: 2 Date: 15 July 2008

Author's response to reviews: see over
Dear Dr. Dunckley,

I would like to thank you and reviewers for the time and thought put into reviewing our manuscript.

MS: 1379402333190286
EGFR and HER2 expression in primary cervical cancers and corresponding metastases: Implications for targeted radiotherapy

We have made necessary changes as suggested by your email of 26 June with reviewers’ comments, as described below.

Referee 1

Discretionary Revisions:

1. It would be more useful and appealing for the readers if the title should include the sites of metastases analysed (i.e.: “and corresponding lymphnode metastases”)

Answer:
Agreed, we have followed the advice by the reviewer. The site of metastases, lymph node, has been added in the title:
EGFR and HER2 expression in primary cervical cancers and corresponding lymph node metastases: Implications for targeted radiotherapy

2. The question posed by the authors seems well defined and the methods be considered partially appropriate. It is in fact unknown whether EGFR immunostaining is to be considered a predictive factor for anti-EGFR treatment in cervical tumours and therefore the technical limitations of immunohistochemistry in determining the biological activity of the EGFR should be mentioned.

Answer:
We have followed the advice by the reviewer and added text discussing the methods used to determine the biological activity of the EGFR. See page 5, last 3 lines, and page 6, paragraph 1.
EGFR expression levels by means of immunohistochemistry (IHC) did not correlate with tumor response and disease control in a recent phase II trial reported by Goncalves et al. This is not unexpected, since EGFR expression does not necessarily correlate with EGFR receptor activation. Other molecular alterations, such as EGFR gene amplification, mutations of the tyrosine kinase domain, and EGFR phosphorylation status, might be useful indicators for the response to EGFR signaling inhibition. However, in the case of targeted radionuclide therapy, tumor cells are mainly killed with delivered radiation and therapeutic efficiency is only dependent on the receptor expression and not whether the receptor function can be blocked or not. Thus, receptor overexpression, by means of IHC, is considered necessary for the success of targeted radiotherapy.

3. It is interesting to note that an apparent lack of concordance for EGFR status is detected in 15 cases. This proportion should not be considered irrelevant to study conclusions especially when we consider that the level of immunohistochemical EGFR expression may not be necessarily related to anti-EGFR treatment outcome.

Answer:
Although 15 changes were observed, only 4 patients with EGFR overexpression (2+/3+) in the primary tumor changed to 1+ or 0 in the corresponding lymph node metastases. In another two patients, EGFR overexpression was gained in lymph node metastases while the primary tumors had low scores. In the case of targeted radiotherapy, receptor overexpression is considered necessary for the success of targeted radiotherapy.

4. Moreover although authors findings may be considered original the number of the cases analysed should prevent any definitive conclusions, we then believe that discussion and conclusions should be re-arranged in this view.

Answer:
The discussion and conclusions have been re-arranged. See page 14, last paragraph, and page 16, last paragraph, line 4-6. Corresponding changes have, of course, been made in the Abstract, conclusions, page 3.

5. If more than one metastatic lymphnode has been analysed for EGFR expression (as it appears from the paper) it would be interesting to know if EGFR pattern is uniformly expressed among different metastatic lymphnodes.

Answer:
Unfortunately, we got only one section with lymph node metastasis from each patient, not all the sections with lymph node metastasis are available (due to the difficulty in getting samples for research). In 11 cases, 2 or more metastatic lymph nodes could be seen on the stained section. Uniform EGFR expressions between/among different metastatic lymph nodes were seen in 10 of the 11 cases (in 3 cases, all metastatic lymph nodes were scored as 3+; in 4 cases, all metastatic lymph nodes were scored as 2+; in another 3 cases, all metastatic lymph nodes were scored as 1+). In the other case, 2 metastatic lymph nodes could be seen, one was scored as 1+, and the other was negatively stained.

Referee 2

Minor comments:

i) Table 1. I think it is unnecessary to describe the distribution of tumour characteristics which have no variance like histology and the presence of ovarian metastases, this can be written in the text.

Answer:
We have followed the advice by the reviewer. Items like histology and ovarian metastases have been removed from Table 1, and have been replaced by text. See page 8, line 15-17.

ii) I think the authors should discuss the problem of using immunohistochemistry in a quantitative way. These problems can account for part of the discrepancies between primaries and metastases

Answer:
We have followed the advice by the reviewer and added a paragraph discussing the problem of using immunohistochemistry in a quantitative way. See page 14, line 1-14.

iii) In speculation of using EGFR for targeted radiotherapy, the authors should discuss the possible side-effects caused by EGFR expression in normal tissue

Answer:
We have followed the advice by the reviewer and added a paragraph discussing the possible side effects using EGFR for targeted radiotherapy. See page 15, paragraph 1.
…… It might be possible to minimize the toxicity and improve therapeutic efficiency by using suitable targeting agents with low uptake in critical normal tissues, and suitable biodistribution. EGFR targeted radiotherapy might also be possible if a tumor and its metastases have a strong EGFR expression to ensure higher tumor uptake than in most normal tissues or local delivery of the targeting agent can be made.

We thank the reviewers once again for constructive advices that helped to improve the manuscript.

I hope the amendment meets with your approval and look very much forward to your favorable reply.

With warm regards,

Yours Sincerely

Qichun Wei, MD, PhD

Deputy Director, Department of Radiation Oncology
The Second Affiliated Hospital
Ministry of Education Key Laboratory of Cancer Prevention and Intervention
Zhejiang University School of Medicine