Author's response to reviews

Title: Prognostic significance of composite expressions of MCM2, Ki-67 and gelsolin in non-small cell lung cancer

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Author's response to reviews: see over
Dear Editors and Reviewers:

Thank you for your time and effort reviewing our manuscript. We greatly appreciate the reviewers’ thorough review and constructive comments. According to the comments, we have performed additional statistical analyses, revised the text, and updated the tables and figures. We believe the revised manuscript has been substantially improved based on the reviewers’ suggestions. Here we outlined our point-by-point responses and revisions as follows.

Reviewer: Dr. Cheng-long L Huang

**Major Compulsory Revisions:**

1. Relationship between expression of MCM2 and Ki-67 and tumor status

   **Response and Revision:** We appreciate Dr. Huang’s input and agree that it is very important to evaluate the relationship between expression of MCM2 and Ki-67 and tumor status. Actually we did analyze the relationship between expression of MCM2 and Ki-67 and tumor status and the results were presented in Table 2 in the original manuscript, where the relationship of major epidemiological and clinicopathological information including tumor status and MCM2, Ki-67 and gelsolin (individual but not combined) was analyzed. We performed analyses for combined MCM2 and Ki-67 and tumor status (stage, grade and histology) and found higher percentage of advanced tumor stage (stage IIIA) for higher expressions of MCM2 and Ki-67 (p=0.06). No apparent relationship between combined MCM2 and Ki-67 and grade and histology. Due to the limited space, we did not present this result in Table 2. To reduce confusion, we changed the title of Table 2 to “**Association Between MCM2, Ki-67, and Gelsolin and Clinicopathological Characteristics in 128 Patients with NSCLC, RPCI, 1995-1999**”.

2. Relationship between gelsolin and nodal status

   **Response and Revision:** We supplemented the analyses for the relationship between MCM2, Ki-67 and gelsolin and nodal status and presented the results in Table 2.

3. Vital status

   **Response and Revision:** We deleted vital status from Table 2 and related text in Page 11 Lines 15-16. We keep vital status in Table 1 because it does not involve any prognostic analysis but just displays patients’ clinicopathological characteristics.

4. Survival time and MCM2, Ki-67 and gelsolin
5. Correlation between MCM2, Ki-67 and gelsolin

**Response and Revision:** We revised the sentence to “Correlation analysis showed that MCM2 was significantly correlated with Ki-67 (R=0.24, p=0.005), and MCM2 and Ki-67 were not significantly correlated with gelsolin” (Page 11, Lines 18-20). We keep the correlations of MCM2/Ki-67 and gelsolin just in case that readers question if we performed this analysis and what the correlation looks like.

6. Composite survival analysis of MCM2, Ki-67 and gelsolin

**Response and Revision:** We appreciate and value Dr. Huang’s comments and suggestions in this portion. We examined the data and realized that the worse prognosis that Dr. Huang mentioned was very likely due to the small sample sizes (n=6 and n=3). Thus we revised the paragraph to make the statement more clear. We replaced the sentence for high gelsolin and low Ki-67 with this one: Although high gelsolin and low Ki-67 showed the highest risk of death, the estimate might be unreliable due to the small number of patients in this analysis (n=6) (Page 12, Lines 15-16 in the revised manuscript). In addition, we supplemented a sentence after the statement for the three markers: Similarly, although high gelsolin, low Ki-67 and low MCM2, as well as high gelsolin, low Ki-67 and high MCM2 conferred higher risk of death, these estimates were unreliable due to the small sample sizes (n=3 for both categories) (Page 12, Lines 19-21 in the revised manuscript). We did not performed additional multivariate analysis because results in Table 3 were from multivariate Cox regression models and we do not think other multivariate analyses without incorporating both vital status and survival time would fit in this analysis. We appreciate Dr. Huang’s insight for the survival curves and have showed the curves for all subgroups in Figure 2, except for the composite variable of MCM2, Ki-67 and gelsolin, because there are 8 subgroups and the curves are too packed to label and read. We regard survival curves are supplement to the Cox models, which show the prognostic value of each subgroup (Table 3).

7. Citation of Ki-67 and NSCLC papers

**Response:** During literature review, we noticed that the prognostic significance of Ki-67 in NSCLC had been studied in a number of clinical studies, and several excellent review papers had been published summarizing the prognostic value of Ki-67 in NSCLC. In the manuscript, we cited a recent review paper, which nicely reviewed 16 studies on Ki-67 and NSCLC, to give readers an overview in this topic (Page 14, Lines 12-15 in the revised manuscript). That is why we did not cite individual studies.

**Minor Essential Revisions:**

1. Method is too long and needs to be shortened (Page 7)
**Response and Revision:** We shortened the Method in page 7 as Dr. Huang suggested.

2. Immunohistochemistry scoring for gelsolin (Page 9, line 1)

   **Response:** Gelsolin staining was initially scored by a pathologist (DFT), with the assistance of an investigator (JY, not a pathologist). Since gelsolin scoring was performed by only one pathologist, we examined the reliability of this scoring by having two pathologists (DFT and JG) independently review slides from approximately half of the cases by random selection. This examination showed 81% agreement between these two pathologists (**Page 9, lines 3-5 in the revised manuscript**).

3. Description of patient characteristics needs to be shortened (Pages 10-11)

   **Response and Revision:** We shortened the description of patient characteristics in pages 10-11 as Dr. Huang suggested.

4. Missing labels on Figure 1 (Page 11, lines 4-6)

   **Response and Revision:** Labels have been added to Figure 1.

5. Inconsistency of 47% and 61.7% in text and Table 1 (Page 11, line 6)

   **Response and Revision:** 61.7% is correct and we have made this correction in the text (**Page 10, line 23 in the revised manuscript**).

6. Low Ki-67 or high Ki-67 (Page 12, line 15)

   **Response and Revision:** Low Ki-67 is correct, but there is a typo for the point estimate, the correct one should be 3.36. We have made this correction (**Page 12, lines 9-10 in the revised manuscript**).

**Discretionary Revisions:** Stratified analyses by stage

**Response:** Dr. Huang has raised a very good suggestion, but due to the small sample size, we noticed stratified analyses by stage generated unreliable results, particularly in multivariate analyses, therefore we did not present the results from stratified analyses in this manuscript.

**Reviewer: Dr. Anne-Pascale Meert**

**Major Compulsory Revisions:** None

**Minor Essential Revisions:**

1. The cut-off point for Ki-67 and MCM2
Response: We thank Dr. Meert for the review and for this insightful suggestion. During the scoring for Ki-67 and MCM2, we classified if a tumor belonged to one of the following 5 categories for Ki-67 and MCM2: negative, <25%, 25-49%, 50-74%, and >=75%. This was based on the commonly used approaches for Ki-67 and MCM2. We did not record the percentage for each tumor and therefore were not able to examine the median values as cut-off points. Our data are consistent with the published data in terms of the distribution of Ki-67 and MCM2 in the above 5 categories, thus we regard the use of 25% as a cut-off point was appropriate in this analysis.

2. Discordances between the text describing phenotypic expression on page 11 and the explanation given on figure 1.

Response and Revision: We have corrected the discordances as: negative expression (C and E), low expression (A and F), and high expression (B, D and G), respectively (Page 10, lines 22-23 in the revised manuscript).

3. Percentage of high MCM2 expression (page 11 and Table 1)

Response and Revision: We have corrected the percentage (Page 10, line 23 in the revised manuscript).

4. The origin of MIB1 antibody

Response and Revision: We checked the origin of MIB1 antibody and confirmed that the origin was Immunotech Inc. We have corrected the inconsistency (Page 7, line 23 in the revised manuscript).

Discretionary Revisions:

1. Patients excluded from the analysis

Response: We have specified the 93 patients who were excluded from the analysis: 53 due to inadequate tumor specimen or poor staining, and 40 were excluded due to causes of death not related to lung cancer (Page 6, lines 15-17 in the revised manuscript).

2. The association between MCM2 expression and tumor histology

Response: We agreed with Dr. Meert that the association between MCM2 expression and tumor histology might be due to the small number of large cell carcinoma and other histology in comparison with adenocarcinomas and squamous cell carcinomas.

Reviewer: Dr. Akiteru Goto

Major Compulsory Revisions:

1. Uniformity of gelsolin expression
Response: We thank Dr. Goto for the review and for the suggestion of including uniformity of gelsolin expression in the analysis. Actually we did analyze uniformity of gelsolin expression and found that patients with variable gelsolin expression pattern, alone or in combination with high levels of MCM2 and Ki-67, was a poor prognostic factor in NSCLC (unadjusted analyses: variable gelsolin & high MCM2 vs. uniform gelsolin & low MCM2: RR=2.12, 95% CI=1.13-3.99; variable gelsolin & high Ki-67 vs. uniform gelsolin & low Ki-67: RR=2.27, 95% CI=1.07-4.82). After controlling for confounders, the strength of association was attenuated to marginal significance, but the trend of association was still obvious. We did not include these results in the manuscript because uniformity of gelsolin expression had been described and analyzed in a previous paper (Lung Cancer 2004; 46:29-42) and because we did not measure the uniformity expression of MCM2 and Ki-67.

2. Result interpretation (Page 11, lines 4-20)

Response and Revision: We agree with Dr. Goto and have revised the result interpretation (Page 11, lines 4-7 in the revised manuscript).

Minor Essential Revisions: None

Discretionary Revisions: None

Again, we greatly appreciate the insightful comments from three reviewers. We have tried our best to address the issues that the reviewers raised, and hope that you find our revised manuscript substantially improved and acceptable for publication in your respected journal of BMC Cancer.

Sincerely,

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