Editors
BMC Cancer

RE: 2nd revised manuscript # 9873564010059150 entitled: “Prognostic significance of composite expressions of MCM2, Ki-67 and gelsolin in non-small cell lung cancer”

Dear Editors and Reviewers:

Again, we greatly appreciate the reviewers’ efforts reading our manuscript in scrutiny and providing very valuable comments. We have revised our manuscript based on Dr. Huang and Dr. Holubkov’s comments, and hereby outline our point-by-point responses and revisions as follows.

Reviewer: Dr. Cheng-long L Huang

Major Compulsory Revisions: Simply results regarding the Ki-67 expression, and briefly discuss this in the paragraph regarding Ki-67

Response and Revision: We thank Dr. Huang for the insightful input regarding the prognostic effect of Ki-67. We re-assessed the Ki-67 data and simplified Ki-67 results as the follows:

1) Revised results regarding Ki-67 (Abstract; Text body: Pages 11-12 in the revised manuscript)
2) Revised Figure 2 (the new Figure 2 now contains survival curves for MCM2, Ki-67, and gelsolin separately, and a curve for MCM2 and gelsolin combined)
3) Eliminated results from the composite analyses involving Ki-67 in Table 3
4) Revised discussion regarding Ki-67 (Pages 13-14)
5) Revised summary paragraph accordingly (Page 15)

Minor Essential Revisions: Relationship between MCM2 and Ki-67 and tumor size

Response and Revision: We have added results for the relationship between MCM2 and Ki-67 and tumor size in Table 2, along with the relationship of gelsolin and tumor size. We did not find significant associations between these three proteins and tumor size, though MCM2 tended to relate to tumor size (p=0.19).

Discretionary Revisions: None

Reviewer: Dr. Richard Holubkov

Major Compulsory Revisions: None

Minor Essential Revisions:

1. p.10: how many of 128 patients were alive at last contact?
**Response and Revision:** 49 patients were alive at the last contact (December 30, 2003). We have added this in **Page 10, the last line in the revised manuscript**.

2. About mean survival time

**Response and Revision:** We appreciate Dr. Holubkov’s valuable comments for this and other statistical issues. We agree with Dr. Holubkov that a simple analysis and presentation of mean survival time does not make a lot of sense, therefore we deleted the description for mean survival time (**Page 11, Lines 8-11 in the 1st revised manuscript**).

3. p. 11: correlation measurement

**Response and Revision:** We looked into Dr. Holubkov’s comment and the previous comment from Dr. Huang on this issue. We agree with Dr. Holubkov and Dr. Huang that a measurement of correlation is not very informative, particularly when MCM2, Ki-67 and gelsolin were measured as binary variables. We have deleted the correlation description for MCM2, Ki-67 and gelsolin (**Page 11, Lines 12-14 in the 1st revised manuscript**).

4. p. 11: Distributions of levels of MCM2, Ki-67, and gelsolin

**Response:** Dr. Holubkov raised very good point in this issue. We agree with Dr. Holubkov that plot presentation for continuous variables would be helpful and informative. In this study, MCM2 and Ki-67 were recorded as binary categorical variables when the pathologists read and scored the tumor specimens. These two biomarkers were not measured as continuous variables initially and were categorized later on. For gelsolin, it was measured as a continuous variable when we generated “gelsolin index” based on the percentage of tumor cells showing immunostaining in 4 intensities (0, 1+, 2+, and 3+). We did examine these data to detect outliers and exclude measurement error by plots. We view this process a necessity in data cleaning and management, but because these plots were somehow complex and were not directly related to the prognostic analysis, we did not present these plots as main results in the manuscript.

5. Table 2: Chi-square analysis for smoking status, stage and grade, which were measured as ordinal variables.

**Response and Revision:** We appreciated Dr. Holubkov’s expertise in this issue, and have re-analyzed these data using Kendall's tau-b test in SPSS. None of the analyses was significant, though Ki-67 and grade was marginally significant as Dr. Holubkov mentioned. We revised the Statistical Methods accordingly (**Pages 9-10 in the revised manuscript**).

6. pp. 11-12: proportional hazards assumption

**Response and Revision:** We checked again the proportional hazards assumption for MCM2, Ki-67 and gelsolin, and noticed the hazard function was proportional for MCM2 and gelsolin, but non-proportional for Ki-67 over time. We might have treated Ki-67 as a covariate instead of strata variable in the initial examination in Cox model, which was incorrect in checking the proportional hazards assumption and thus failed to show the violation of assumption for Ki-67. Based on this observation, we separated the analysis of MCM2 and gelsolin from Ki-67. We
used Cox Proportional Hazards Regression for MCM2 and gelsolin, and used the time-dependent Cox regression for Ki-67. Due the these changes, the following revisions were made:

1. Revised Title (deleted “composite expressions”).
2. Revised Statistical Methods (Page 10, Lines 2-10 in the revised manuscript).
3. Deleted results involving Ki-67 using Cox Proportional Hazards Regression; added RR and 95% CI for Ki-67 based on the time-dependent Cox regression (Table 3).

Discretionary Revisions:

1. The selection of these three biomarkers

**Response:** Initially, these three biomarkers were studied independently by different researchers at the Roswell Park Cancer Institute for their specific research interests, using the same bank of patient data. As the research progressed, we communicated and established collaborations among these researchers and ended up with this composite analysis. As described in the Introduction and Discussion, MCM2 and Ki-67 are two important proliferation markers, and gelsolin is an important cell motility-mediating marker. In fact, there are a large number of proteins and molecules involved in the pathways of proliferation and motility-mediation. An ideal study is such one that can include as many biomarkers as possible and investigate the biomarker-disease relationship based on pathway-driven mechanism. But due to the resource restraint, many investigators have to choose some representative biomarkers in their studies. Findings from these studies (which are usually small and limited) are actually important for the design of future large scale studies and consortium studies.

2. p.10: Repeat non-key percentages from Tables 1 and 2 in the text of the Results section.

**Response:** The current description of patient characteristics in the text is based on the comments from the 3 reviewers in the first review. We think some description may be helpful to readers when they read through the tables.

3. About statistical modeling and interaction test

**Response:** We followed Dr. Holubkov’s suggestion and performed model testing for MCM2 and gelsolin (Ki-67 was not included because it was not a significant prognostic factor and it did not fit the Cox Proportional Hazards Regression). The testing showed that MCM2-gelsolin model was better than MCM2 but equivalent to gelsolin model. We further performed an interaction test for MCM2 and gelsolin and did not find a significant interaction between MCM2 and gelsolin. Interestingly, MCM2 and gelsolin alone was not a significant factor in the interaction test. While statistical modeling is helpful and instrumental, we think data interpretation should take into account the biological mechanisms in biomedical research.

4. Discussion about the limited sample size.

**Response and Revision:** We added some discussion about the limited sample size in the Discussion section (Pages 13-14 in the revised manuscript).
Once again, we greatly appreciate the insightful comments from Drs. Huang and Holubkov, and two previous reviewers. We have tried our best to improve the manuscript by addressing the relevant scientific issues. We believed this 2nd revised manuscript has been substantially improved based on the comments from all reviewers, and hope that this manuscript will be accepted for publication soon in *BMC Cancer*. Thanks again for your hard work and efforts.

Sincerely,

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